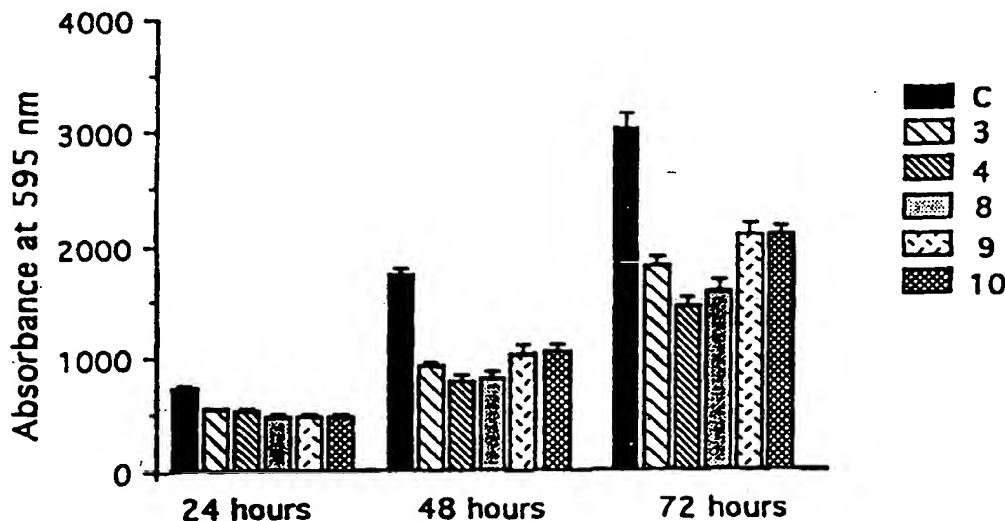




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(54) Title: PITUITARY-TUMOR-TRANSFORMING-GENES, AND RELATED PRODUCTS



(57) Abstract

Polypeptides are expressed by the pituitary-tumor-transforming-gene (PTTG), formerly known as pituitary-tumor-specific-gene (PTSG), and nucleic acids encoding them. Examples are the human and rat PTTG proteins. The nucleic acids may be applied to the production of a recombinant protein, and to the detection of the presence of PTTG genes in different species. The nucleic acids may be operatively linked to a vector, optionally provided with control and expression sequences and/or being carried by a host cell. The nucleic acids may also be delivered to a mammal to compensate for the absence, or a defective expression, of endogenous protein. The nucleic acids, proteins, and antibodies are also employed in diagnostic assays, as well as, for example, in the production of anti-PTTG antibodies (protein), therapeutic compositions and other applications of the proteins and antibodies. Various kits utilize nucleic acids, polypeptides, and/or antibodies. A transgenic non-human mammal expresses PTTG.

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PITUITARY-TUMOR-TRANSFORMING-GENES, AND RELATED PRODUCTS

BACKGROUND OF THE INVENTION

5 This invention was made at least in part with United States Government support under Grant Number DK42742, awarded by the National Institutes of Health. The Government may have certain rights in this invention.

This application claims the priority of the filing date of US Provisional Application Serial No. 60/031,338, entitled NUCLEIC ACID ENCODING A FAMILY OF PITUITARY-TUMOR-
10 SPECIFIC-GENES, AND PRODUCTS RELATED THERETO, by Shlomo Melmed and Lin Pei, filed November 21, 1996.

Field of the Invention

The present invention relates to nucleic acids and proteins encoded thereby. Invention nucleic acids encode a novel family of pituitary-tumor-specific-gene proteins. The invention also
15 relates to methods for making and using such nucleic acids and proteins.

Description of the Background

Cancers and tumors are the second most prevalent cause of death in the United States, causing 450,000 deaths per year. One in three Americans will develop cancer, and one in five will die of cancer (Scientific American Medicine, part 12, I, 1, section dated 1987). While substantial
20 progress has been made in identifying some of the likely environmental and hereditary causes of cancer, the statistics for the cancer death rate indicates a need for substantial improvement in the therapy for cancer and related diseases and disorders.

A number of cancer genes, i.e., genes that have been implicated in the etiology of cancer, have been identified in connection with hereditary forms of cancer and in a large number of well-
25 studied tumor cells. Study of cancer genes have helped provide some understanding of the process of tumorigenesis. While a great deal more remains to be learned about cancer genes, the presently known cancer genes serve as useful models for understanding tumorigenesis.

Cancer genes are broadly classified into "oncogenes" which, when activated, promote tumorigenesis, and "tumor suppressor genes" which, when damaged, fail to suppress tumorigenesis.
30 While these classifications provide a useful method for conceptualizing tumorigenesis, it is also possible that a particular gene may play differing roles depending upon the particular allelic form of that gene, its regulatory elements, the genetic background and the tissue environment in which it is operating.

Tumor suppressor genes are genes that in their wild-type alleles, express proteins that
35 suppress abnormal cellular proliferation. When the gene coding for a tumor suppressor protein is

mutated or deleted, the resulting mutant protein or the complete lack of tumor suppressor protein expression may fail to correctly regulate cellular proliferation, and abnormal cellular proliferation may take place, particularly if there is already existing damage to the cellular regulatory mechanism. A number of well-studied human tumors and tumor cell lines have been shown to have
5 missing or nonfunctional tumor suppressor genes. Examples of tumor suppression genes include, but are not limited to the retinoblastoma susceptibility gene or RB gene, the p53 gene, the deleted in colon carcinoma (DDC) gene and the neurofibromatosis type 1 (NF-1) tumor suppressor gene. Loss of function or inactivation of tumor suppressor genes may play a central role in the initiation and/or progression of a significant number of human cancers.

10 Anterior pituitary tumors are mostly benign hormone-secreting or non-functioning adenomas arising from a monoclonal expansion of a genetically mutated cell. Pathogenesis of tumor formation in the anterior pituitary has been intensively studied. Mechanisms for pituitary tumorigenesis involve a multi-step cascade of recently characterized molecular events. The most well characterized oncogene in pituitary tumors is *gsp*, a constitutively active G_{α} resulting from
15 activating point mutations in this gene.

G_{α} mutations occur in about 40% of GH-secreting tumors, and constitutively activated CREB is also found in a subset of these tumors. Although the importance of G_{α} mutant proteins in the development of growth-hormone secreting pituitary tumors is well established, only about one third of these tumors contains these mutations, indicating the presence of additional transforming
20 events in pituitary tumorigenesis. Although point mutations of Ras oncogene, loss of heterozygosity (LOH) near the Rb locus on chromosome 13, and LOH on chromosome 11 have been implicated in some pituitary tumors, the mechanism that causes pituitary cell transformation remains largely unknown. Thus, there is a need in the art for additional pituitary derived proteins that are associated with pituitary cell transformation.

25 SUMMARY OF THE INVENTION

The present invention relates to isolated, purified Mammalian-pituitary-Transforming-Gene (PTTG) proteins, formerly named Mammalian Pituitary-Tumor-Specific-Gene (PTSG) proteins. The PTTG proteins of the invention and fragments thereof, are useful in bioassays, as immunogens for
30 producing anti-PTTG antibodies, or in therapeutic compositions containing such proteins and/or antibodies.

This invention also relates to a transgenic non-human mammal that expresses PTTG protein.

The present invention also relates to isolated nucleic acids encoding PTTG (PTSG) proteins of mammalian origin, such as human, rat, etc. The PTTG encoding nucleic acid is also provided in the form of a vector carrying it, as hybridizing probes/primers, in host cells carrying them, as
35 anti-sense oligonucleotides, in DNA and RNA forms, and related compositions. The nucleic acid molecules described herein may be incorporated into expression systems known to those of skill in

the art. The PTTG nucleic acids are useful as probes for assaying for the presence and/or amount of a PTTG gene or mRNA transcript in a given sample. The nucleic acid molecules described herein, and oligonucleotide fragments thereof, are also useful as primers and/or templates in a PCR reaction for amplifying genes encoding PTTG proteins.

Antibodies that are immunoreactive with invention PTTG proteins are also provided. These antibodies are useful in diagnostic assays to determine levels of PTTG proteins present in a given sample, e.g., tissue samples, biological fluids, Western blots, and the like. The antibodies can also be used to purify PTTG proteins from crude cell extracts and the like. Moreover, these antibodies are considered therapeutically useful to counteract or supplement the biological effect of PTTGs in vivo.

Methods and diagnostic systems for determining the levels of PTTG protein in various tissue samples are provided as well. These diagnostic methods can be used for monitoring the level of therapeutically administered PTTG protein or fragments thereof to facilitate the maintenance of therapeutically effective amounts. These diagnostic methods can also be used to diagnose pathologic and physiological disorders that result from abnormal levels of PTTG protein.

BRIEF DESCRIPTION OF THE FIGURE

Figure 1 shows the effect of PTTG expression on cell proliferation. The cell growth rate is expressed as absorbance at 595 nM. The error bars represent SEM (n=6). Three independent experiments were performed.

DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE INVENTION

The present invention arose from a desire by the inventors to improve over the prior art methods for detection of diseases or conditions associated with overproduction of, or abnormalities in, PTTG expression. The inventors set out to isolate a PTTG gene in one species and then utilized probes based on the thus found gene sequence to probe the genomic DNA of other species, particularly human, and devise a method for mutating the PTTG gene as well as a method for replacing defective PTTG genes. They, in addition, conceived of a transgenic animal for use as a model for the study of such diseases and conditions in humans. This invention therefore provides isolated, purified mammalian pituitary-tumor-specific-gene (PTTG) proteins, polypeptides, and fragments thereof encoded by the nucleic acid of the invention. As used herein, the phrase "PTTG" refers to a mammalian family of isolated and/or substantially pure proteins, preferably human, that are able to transform cells in tissue culture, e.g., NIH 3T3 cells, and the like. The PTTG proteins of the invention have the ability to induce tumor formation in nude mice, e.g., when transfected into NIH 3T3 cells, and the like. The PTTG proteins of the invention include naturally occurring allelic variants thereof encoded by mRNA generated by alternative splicing of a primary transcript, and further include fragments which retain at least one native biological activity, such as immunogenicity.

To identify genes specifically expressed in pituitary tumor cells, the inventors utilized GH-secreting and prolactin-secreting rat pituitary tumor cell lines. In this manner, they utilized them to eliminate the admixture of normal tissues present in surgically excised human pituitary tumors and solid experimental rat tumors. Upon screening about 30% of expressed mRNA, a pituitary tumor transforming gene (PTTG) was identified and characterized. The sequence of PTTG revealed no homology to any known sequences in the GenBank. The PTTG gene encodes a protein of 199 amino acids that contains no characterized functional motif, which clearly indicates that PTTG is a novel protein.

The inventors showed the pituitary tumor specific expression of PTTG by Northern blot analysis. Other than pituitary tumor cells, testis tissue is the only normal non-tumor tissue to show PTTG expression. Interestingly, the PTTG messenger RNA in testis appears to be about 300 bp shorter than that of the pituitary tumor, which indicates that the testis messenger is a PTTG splice variant of the pituitary messenger..

The importance of PTTG in tumorigenesis is illustrated by its ability to transform 3T3 fibroblasts when overexpressed in these cells, as shown by morphological change and anchorage-independent growth of PTTG transfectants in soft agar. This finding, moreover, is underscored by the discovery that multiple tumor cell lines express abundant amounts of PTTG. Furthermore, nude mice injected with PTTG-expressing 3T3 cells developed large tumors within 3 weeks at all injection sites. These data show that PTTG alone is capable of cellular transformation without the requirement of a complimentary oncogene, and that it is potently tumorigenic in vivo. In general, full cell transformation requires two complementary oncogenes. See, Land et al, Nature, 304:696 (1983); Schwab et al., Nature, 316:160 (1985); Ruley et al., Nature 304:602 (1983). In some cases, however, the overexpression of a single oncogene may be sufficient to induce cellular transformation, as is the case in the Rat-1 cell transformation by overexpression of the Ras gene alone. See, Reynolds, V.L. Oncogene, 1:323 (1987). In the present case, the inventors found that PTTG does not stimulate cell proliferation in cultured transfected cells within 72 hours of assaying time. In fact, they found that PTTG unexpectedly inhibits all proliferation in cultured transfected cells. This anti-proliferative effect is similar to the potent inhibition of cell growth seen by Massague et al. with TGF β . See, Massague et al., Ann. Rev. Cell Biol. 6:597 (1990). Once the cells are transformed, however, cell proliferation is accelerated, and rapid growth of tumors is seen in nude mice.

The PTSG proteins of this invention are polypeptide selectively bound by anti-PTTG (anti-PTSG) antibody, the antibody preferably binding to the human protein, including amino acid sequences SEQ ID NO:2, SEQ. ID No: 4, and fragments 5 to 50 amino acids long which bind to anti-PTTG antibody. The isolated PTSG proteins of the invention are generally free of other cellular components and/or contaminants normally associated with a native in vivo environment,

although they may have a certain content of these products, such as proteins, RNA, DNA and polysaccharides..

The PTTG proteins are primarily, although not exclusively, expressed by pituitary tumor cells with expression detected in testis. The transcript in rat pituitary tumor cells is about 1.3 kb in size while the transcript in testis is about 1 kb, as observed by a Northern blot assay. Splice variant cDNA transcripts encoding a PTTG family of proteins are clearly also contemplated by the present invention.

Use of the terms "isolated" and/or "purified" in the present specification and claims as a modifier of DNA, RNA, polypeptide or proteins means that the DNA, RNA, polypeptide or proteins so designated have been produced in such form by the hand of man, and thus are separated from their native in vivo cellular environment. As a result of this human intervention, the recombinant DNAs, RNAs, polypeptide and proteins of the invention are useful in ways described herein that the DNAs, RNAs, polypeptide or proteins as they naturally occur are not.

As used herein, "mammalian" refers to the variety of species from which the PTTG protein of the invention is derived from, e.g., human, rat, mouse, rabbit, monkey, baboon, bovine, porcine, ovine, canine, feline, and the like. A preferred PTTG protein herein, is human PTTG.

Also part of this invention is the PTTG gene, which when defective or present, is responsible for pituitary tumorigenesis. A search of GenBank and protein profile analysis (BLAST Program search of databases of the national center for Biotechnology Information) indicated that PTTG shares no homology with known sequences, and its encoded protein is highly hydrophilic, and contains no well recognized functional motifs.

Presently preferred PTTG proteins of the invention include amino acid sequences that are substantially the same as the amino acid sequence SEQ ID NO:2, SEQ. ID No: 4, and fragments thereof about 5 to 50 amino acids long, as well as biologically active, modified forms thereof. Those of skill in the art will recognize that numerous residues of the above-described sequences can be substituted with other, chemically, sterically and/or electronically similar residues without substantially altering the biological activity of the resulting receptor species. In addition, larger polypeptide sequences containing substantially the same sequence as SEQ ID NO:2 therein (e.g., splice variants) are contemplated.

As employed herein, the term "substantially the same amino acid sequence" refers to amino acid sequences having at least about 70% identity with respect to the reference amino acid sequence, and retaining comparable functional and biological activity characteristic of the protein defined by the reference amino acid sequence. Preferably, proteins having "substantially the same amino acid sequence" will have at least about 80%, more preferably 90% amino acid identity with respect to the reference amino acid sequence; with greater than about 95% amino acid sequence identity being especially preferred. It is recognized, however, that polypeptide (or nucleic acids referred to

hereinbefore) containing less than the described levels of sequence identity arising as splice variants or that are modified by conservative amino acid substitutions, or by substitution of degenerate codons are also encompassed within the scope of the present invention.

5 The term "biologically active" or "functional", when used herein as a modifier of the PTTG protein(s) of this invention or polypeptide fragment thereof, refers to a polypeptide that exhibits at least one of the functional characteristics attributed to PTTG. For example, one biological activity of PTTG is the ability to transform cells in vitro (e.g., NIH 3T3 cells, and the like). Yet another biological activity of PTTG is the ability to induce tumor formation in nude mice (e.g., when transfected into NIH 3T3 cells, and the like).

10 PTTG is also active as an immunogen for the production of polyclonal and monoclonal antibodies that bind selectively to PTTG. Thus, an invention nucleic acid encoding PTTG will encode a polypeptide specifically recognized by an antibody that also specifically recognizes the PTTG protein, preferably human, including amino acid sequences SEQ ID NO:2, SEQ. ID No: 4, and fragments thereof about 5 to 50 amino acids long which bind to anti-PTTG antibody. Such
15 activity may be assayed by any method known to those of skill in the art. For example, a test-polypeptide encoded by a PTTG cDNA may be used to produce antibodies, which are then assayed for their ability to bind to the protein including the sequence set forth in SEQ ID NO:2. If the antibody binds to the test-polypeptide and the protein including the sequence set forth in SEQ ID NO:2 with substantially the same affinity, then the polypeptide possesses the requisite biological
20 activity.

The PTTG proteins of the invention may be isolated by methods well-known in the art, e.g., the recombinant expression systems described herein, precipitation, gel filtration, ion-exchange, reverse-phase and affinity chromatography, and the like. Other well-known methods are described in Deutscher et al., Guide to Protein Purification: Methods in Enzymology Vol. 182, (Academic
25 Press, (1990)), which is incorporated herein by reference. Alternatively, the isolated polypeptide of the present invention can be obtained using well-known recombinant methods as described, for example, in Sambrook et al., supra., 1989).

An example of the means for preparing the invention polypeptide(s) is to express nucleic acids encoding the PTTG in a suitable host cell, such as a bacterial cell, a yeast cell, an amphibian
30 cell (i.e., oocyte), or a mammalian cell, using methods well known in the art, and recovering the expressed polypeptide, again using well-known methods. The PTTG polypeptide of the invention may be isolated directly from cells that have been transformed with expression vectors as described herein. The invention polypeptide, biologically active fragments, and functional equivalents thereof can also be produced by chemical synthesis. For example, synthetic polypeptide can be produced
35 using Applied Biosystems, Inc. Model 430A or 431A automatic peptide synthesizer (Foster City, CA) employing the chemistry provided by the manufacturer.

Also encompassed by the term PTTG are polypeptide fragments or polypeptide analogs thereof. The term "polypeptide analog" includes any polypeptide having an amino acid residue sequence substantially identical to a sequence specifically shown herein in which one or more residues have been conservatively substituted with a functionally similar residue and which displays the ability to mimic PTTG as described herein. Examples of conservative substitutions include the substitution of one non-polar (hydrophobic) residue such as isoleucine, valine, leucine or methionine for another, the substitution of one polar (hydrophilic) residue for another such as between arginine and lysine, between glutamine and asparagine, between glycine and serine, the substitution of one basic residue such as lysine, arginine or histidine for another, or the substitution of one acidic residue, such as aspartic acid or glutamic acid for another. The phrase "conservative substitution" also includes the use of a chemically derivatized residue in place of a non-derivatized residue, provided that such polypeptide displays the requisite binding activity.

"Chemical derivative" refers to a subject polypeptide having one or more residues chemically derivatized by reaction of a functional side group. Such derivatized molecules include, for example, those molecules in which free amino groups have been derivatized to form amine hydrochlorides, p-toluene sulfonyl groups, carbobenzoxy groups, t-butyloxycarbonyl groups, chloroacetyl groups or formyl groups. Free carboxyl groups may be derivatized to form salts, methyl and ethyl esters or other types of esters or hydrazides. Free hydroxyl groups may be derivatized to form O-acyl or O-alkyl derivatives. The imidazole nitrogen of histidine may be derivatized to form N-im-benzylhistidine. Also included as chemical derivatives are those peptides which contain one or more naturally occurring amino acid derivatives of the twenty standard amino acids. For example, 4-hydroxyproline may be substituted for proline; 5-hydroxylysine may be substituted for lysine; 3-methylhistidine may be substituted for histidine; homoserine may be substituted for serine; and ornithine may be substituted for lysine. Polypeptide of the present invention also include any polypeptide having one or more additions and/or deletions of residues, relative to the sequence of a polypeptide whose sequence is shown herein, so long as the requisite activity is maintained.

The present invention also provides compositions containing an acceptable carrier and any of an isolated, purified PTTG polypeptide, an active fragment or polypeptide analog thereof, or a purified, mature protein and active fragments thereof, alone or in combination with each other. These polypeptide or proteins can be recombinantly derived, chemically synthesized or purified from native sources. As used herein, the term "acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as phosphate buffered saline solution, water and emulsions such as an oil/water or water/oil emulsion, and various types of wetting agents.

In accordance with another embodiment of the present invention, there are provided isolated nucleic acids, which encode the PTTG (pituitary-tumor-transforming-gene) proteins of the invention,

and fragments thereof. The nucleic acid molecules described herein are useful for producing invention proteins, when such nucleic acids are incorporated into a variety of protein expression systems known to those of skill in the art. In addition, such nucleic acid molecules or fragments thereof can be labeled with a readily detectable substituent and used as hybridization probes for assaying for the presence and/or amount of a PTTG gene or mRNA transcript in a given sample. The nucleic acid molecules described herein, and fragments thereof, are also useful as primers and/or templates in a PCR reaction for amplifying genes encoding the invention protein described herein.

The term "nucleic acid" (also referred to as polynucleotides) encompasses ribonucleic acid (RNA) or deoxyribonucleic acid (DNA), probes, oligonucleotides, and primers. DNA can be either complementary DNA (cDNA) or genomic DNA, e.g. a gene encoding a PTTG protein. One means of isolating a nucleic acid encoding an PTTG polypeptide is to probe a mammalian genomic library with a natural or artificially designed DNA probe using methods well known in the art. DNA probes derived from the PTTG gene are particularly useful for this purpose. DNA and cDNA molecules that encode PTTG polypeptide can be used to obtain complementary genomic DNA, cDNA or RNA from mammalian (e.g., human, mouse, rat, rabbit, pig, and the like), or other animal sources, or to isolate related cDNA or genomic clones by the screening of cDNA or genomic libraries, by methods described in more detail below. Examples of nucleic acids are RNA, cDNA, or isolated genomic DNA encoding an PTTG polypeptide. Such nucleic acids may include, but are not limited to, nucleic acids comprising SEQ ID NO:1, alleles thereof, preferably at least nucleotides 293-889 of SEQ ID NO:1 or splice variant cDNA sequences thereof.

As used herein, the phrases "splice variant" or "alternatively spliced", when used to describe a particular nucleotide sequence encoding an invention receptor, refers to a cDNA sequence that results from the well known eukaryotic RNA splicing process. The RNA splicing process involves the removal of introns and the joining of exons from eukaryotic primary RNA transcripts to create mature RNA molecules of the cytoplasm. Methods of isolating splice variant nucleotide sequences are well known in the art. For example, one of skill in the art may employ nucleotide probes derived from the PTTG encoding DNA of SEQ ID NO:1, SEQ. ID No: 3, alleles thereof, splice variants thereof or fragments thereof about 10 to 150 nucleotide long and their anti-sense nucleic acids to screen the cDNA or genomic library of the same or other species as described herein.

In one embodiment of the present invention, DNAs encoding the PTTG proteins of this invention comprise SEQ. ID NO:1, SEQ. ID No: 3, alleles thereof, splice variants thereof and fragments thereof about 15 to 150 nucleotide long and anti-sense nucleic acids thereof. In another embodiment of the present invention, DNA molecules encoding the invention proteins comprise nucleotides 293-889 of SEQ ID NO:1, alleles thereof, splice variants thereof and fragments thereof

about 10 to 150 nucleotide long. In yet another embodiment, the DNA comprises nucleotides 292-899 of SEQ ID NO:3, alleles thereof, splice variants thereof and fragments thereof about 10 to 150 nucleotides long.

As employed herein, the term "substantially the same nucleotide sequence" refers to DNA having sufficient identity to the reference polynucleotide, such that it will hybridize to the reference nucleotide under moderately stringent hybridization conditions. In one embodiment, DNA having substantially the same nucleotide sequence as the reference nucleotide sequence encodes substantially the same amino acid sequence as that set forth in SEQ ID NO:2, or a larger amino acid sequence including SEQ ID NO:2. In another embodiment, DNA having "substantially the same nucleotide sequence" as the reference nucleotide sequence has at least 60% identity with respect to the reference nucleotide sequence. DNA having at least 70%, more preferably at least 90%, yet more preferably at least 95%, identity to the reference nucleotide sequence is preferred.

The present invention also encompasses nucleic acids which differ from the nucleic acids shown in SEQ ID NO:1, but which have the same phenotype. Phenotypically similar nucleic acids are also referred to as "functionally equivalent nucleic acids". As used herein, the phrase "functionally equivalent nucleic acids" encompasses nucleic acids characterized by slight and non-consequential sequence variations that will function in substantially the same manner to produce the same protein product(s) as the nucleic acids disclosed herein. In particular, functionally equivalent nucleic acids encode polypeptide that are the same as those disclosed herein or that have conservative amino acid variations, or that encode larger polypeptide that include SEQ ID NO:2. For example, conservative variations include substitution of a non-polar residue with another non-polar residue, or substitution of a charged residue with a similarly charged residue. These variations include those recognized by skilled artisans as those that do not substantially alter the tertiary structure of the protein.

Further provided are nucleic acids encoding PTTG polypeptides that, by virtue of the degeneracy of the genetic code, do not necessarily hybridize to the invention nucleic acids under specified hybridization conditions. Preferred nucleic acids encoding the PTTG polypeptide of the invention comprise nucleotides encoding SEQ ID NO:2, SEQ. ID No: 4, and fragments thereof about 5 to 50 amino acids long. Exemplary nucleic acids encoding a PTTG protein of the invention may be selected from the following.

- (a) DNA encoding the amino acid sequence set forth in SEQ. ID No:2,
- (b) DNA that hybridizes to the DNA of (a) under moderately stringent conditions, wherein said DNA encodes biologically active PTTG, or
- (c) DNA degenerate with respect to either (a) or (b) above, wherein said DNA encodes biologically active PTTG.

Hybridization refers to the binding of complementary strands of nucleic acid (i.e., sense:antisense strands or probe:target-DNA) to each other through hydrogen bonds, similar to the

bonds that naturally occur in chromosomal DNA. Stringency levels used to hybridize a given probe with target-DNA can be readily varied by those of skill in the art.

5 The phrase "stringent hybridization" is used herein to refer to conditions under which polynucleic acid hybrids are stable. As known to those of skill in the art, the stability of hybrids is reflected in the melting temperature (T_m) of the hybrids. In general, the stability of a hybrid is a function of sodium ion concentration and temperature. Typically, the hybridization reaction is performed under conditions of lower stringency, followed by washes of varying, but higher, stringency. Reference to hybridization stringency relates to such washing conditions.

10 As used herein, the phrase "moderately stringent hybridization" refers to conditions that permit target-DNA to bind a complementary nucleic acid that has about 60% identity, preferably about 75% identity, more preferably about 85% identity to the target DNA; with greater than about 90% identity to target-DNA being especially preferred. Preferably, moderately stringent conditions are conditions equivalent to hybridization in 50% formamide, 5 x Denhart's solution, 5 x SSPE, 0.2% SDS at 42°C, followed by washing in 0.2 x SSPE, 0.2% SDS, at 65°C.

15 The phrase "high stringency hybridization" refers to conditions that permit hybridization of only those nucleic acid sequences that form stable hybrids in 0.018 M NaCl at 65°C (i.e., if a hybrid is not stable in 0.018 M NaCl at 65°C, it will not be stable under high stringency conditions, as contemplated herein). High stringency conditions can be provided, for example, by hybridization in 50% formamide, 5X Denhart's solution, 5X SSPE, 0.2% SDS at 42°C, followed by washing in 20 0.1 x SSPE, and 0.1% SDS at 65°C.

The phrase "low stringency hybridization" refers to conditions equivalent to hybridization in 10% formamide, 5X Denhart's solution, 6 x SSPE, 0.2% SDS at 42°C, followed by washing in 1 x SSPE, 0.2% SDS, at 50°C. Denhart's solution and SSPE (see, e.g., Sambrook et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1989) are well 25 known to those of skill in the art as are other suitable hybridization buffers.

As used herein, the term "degenerate" refers to codons that differ in at least one nucleotide from a reference nucleic acid, e.g., SEQ ID NO:1 or SEQ. ID No: 3, but encode the same amino acids as the reference nucleic acid. For example, codons specified by the triplets "UCU", "UCC", "UCA", and "UCG" are degenerate with respect to each other since all four of these codons encode 30 the amino acid serine.

Preferred nucleic acids encoding the invention polypeptide(s) hybridize under moderately stringent, preferably high stringency, conditions to substantially the entire sequence, or substantial portions, i.e., typically at least 15-30 nucleotide, of SEQ ID NO:1, SEQ. ID No: 3, although longer fragments are also contemplated.

35 Site-directed mutagenesis of any region of PTTG cDNA is contemplated herein for the production of mutant PTTG cDNAs. For example, the Transformer Mutagenesis Kit (available

from Clontech) can be used to construct a variety of missense and/or nonsense mutations to PTTG cDNA.

The invention nucleic acids can be produced by a variety of methods well-known in the art, e.g., the methods described herein, employing PCR amplification using oligonucleotide primers from various regions of SEQ ID NO:1, and the like.

In accordance with a further embodiment of the present invention, optionally labeled PTTG-encoding cDNAs, or fragments thereof, can be employed to probe library(ies) (e.g., cDNA, genomic, and the like) for additional nucleic acid sequences encoding related novel mammalian PTTG proteins. Construction of mammalian cDNA and genomic libraries, preferably a human library, is well-known in the art. Screening of such a cDNA or genomic library is initially carried out under low-stringency conditions, which comprise a temperature of less than about 42°C, a formamide concentration of less than about 50%, and a moderate to low salt concentration.

Presently preferred probe-based screening conditions comprise a temperature of about 37°C, a formamide concentration of about 20%, and a salt concentration of about 5X standard saline citrate (SSC; 20 x SSC contains 3 M sodium chloride, 0.3 M sodium citrate, pH 7.0). Such conditions will allow the identification of sequences which have a substantial degree of similarity with the probe sequence, without requiring perfect homology. The phrase "substantial similarity" refers to sequences which share at least 50% homology. Preferably, hybridization conditions will be selected which allow the identification of sequences having at least 70% homology with the probe, while discriminating against sequences which have a lower degree of homology with the probe. As a result, nucleic acids having substantially the same, i.e., similar, sequence as the coding region of the nucleic acids of the invention, preferably as nucleotides 293-889 of SEQ ID NO:1 are obtained.

As used herein, a nucleic acid "probe" is single-stranded DNA or RNA, or analogs thereof, that has a sequence of nucleotide that includes at least 14, preferably at least 20, more preferably at least 50, contiguous bases that are the same as (or the complement of) any 14 or more contiguous bases set forth in any of SEQ ID NO: 1 or SEQ. ID No: 3. Preferred regions from which to construct probes include 5' and/or 3' coding regions of SEQ ID NO:1. In addition, the entire cDNA encoding region of an invention PTTG protein, or the entire sequence corresponding to SEQ ID NO:1, may be used as a probe. Probes may be labeled by methods well-known in the art, as described hereinafter, and used in various diagnostic kits.

As used herein, the terms "label" and "indicating means" in their various grammatical forms refer to single atoms and molecules that are either directly or indirectly involved in the production of a detectable signal. Any label or indicating means can be linked to invention nucleic acid probes, expressed proteins, polypeptide fragments, or antibody molecules. These atoms or molecules can be used alone or in conjunction with additional reagents. Such labels are themselves well-known

in clinical diagnostic chemistry.

The labeling means can be a fluorescent labeling agent that chemically binds to antibodies or antigens without denaturation to form a fluorochrome (dye) that is a useful-immunofluorescent tracer. A description of immunofluorescent analytic techniques is found in DeLuca,
5 "Immunofluorescence Analysis", in *Antibody As a Tool*, Marchalonis et al., eds., John Wiley & Sons, Ltd., pp. 189-231 (1982), which is incorporated herein by reference.

In one embodiment, the indicating group is an enzyme, such as horseradish peroxidase (HRP), glucose oxidase, and the like. In another embodiment, radioactive elements are employed labeling agents. The linking of a label to a substrate, i.e., labeling of nucleic acid probes,
10 antibodies, polypeptide, and proteins, is well known in the art. For instance, an invention antibody can be labeled by metabolic incorporation of radiolabeled amino acids provided in the culture medium. See, for example, Galfre et al., *Meth. Enzymol.*, 73:3-46 (1981). Conventional means of protein conjugation or coupling by activated functional groups are particularly applicable. See, for example, Aurameas et al., *Scand. J. Immunol.*, Vol. 8, Suppl. 7:7-23 (1978), Rodwell et al.,
15 *Biotech.*, 3:889-894 (1984), and U.S. Patent No. 4,493,795.

Also provided are antisense oligonucleotides having a sequence capable of binding specifically with any portion of an mRNA that encodes PTTG polypeptide so as to prevent translation of the mRNA. The antisense oligonucleotide may have a sequence capable of binding specifically with any portion of the sequence of the cDNA encoding PTTG polypeptide. As used
20 herein, the phrase "binding specifically" encompasses the ability of a nucleic acid sequence to recognize a complementary nucleic acid sequence and to form double-helical segments therewith via the formation of hydrogen bonds between the complementary base pairs. An example of an antisense oligonucleotide is an antisense oligonucleotide comprising chemical analogs of nucleotide.

Compositions comprising an amount of the antisense oligonucleotide, described above,
25 effective to reduce expression of PTTG polypeptide by passing through a cell membrane and binding specifically with mRNA encoding PTTG polypeptide so as to prevent translation and an acceptable hydrophobic carrier capable of passing through a cell membrane are also provided herein. Suitable hydrophobic carriers are described, for example, in U.S. Patent Nos. 5,334,761; 4,889,953; 4,897,355, and the like. The acceptable hydrophobic carrier capable of passing through
30 cell membranes may also comprise a structure which binds to a receptor specific for a selected cell type and is thereby taken up by cells of the selected cell type. The structure may be part of a protein known to bind to a cell-type specific receptor.

Antisense oligonucleotide compositions are useful to inhibit translation of mRNA encoding invention polypeptide. Synthetic oligonucleotides, or other antisense chemical structures are
35 designed to bind to mRNA encoding PTTG polypeptide and inhibit translation of mRNA and are useful as compositions to inhibit expression of PTTG associated genes in a tissue sample or in a

subject.

In accordance with another embodiment of the invention, kits are provided for detecting mutations, duplications, deletions, rearrangements or aneuploidies in the PTTG gene comprising at least one invention PTTG probe or antisense nucleotide.

5 The present invention provides means to modulate levels of expression of PTTG polypeptide by employing synthetic antisense oligonucleotide compositions (hereinafter SAOC) which inhibit translation of mRNA encoding these polypeptide. Synthetic oligonucleotides, or other antisense chemical structures designed to recognize and selectively bind to mRNA, are constructed to be complementary to portions of the PTTG coding strand or nucleotide sequences shown in SEQ. ID
10 No: 1 or SEQ. ID No: 3. The SAOC is designed to be stable in the blood stream for administration to a subject by injection or by direct tumor site integration, or stable in laboratory cell culture conditions. The SAOC is designed to be capable of passing through the cell membrane in order to enter the cytoplasm of the cell by virtue of physical and chemical properties of the SAOC which render it capable of passing through cell membranes, for example, by designing small, hydrophobic
15 SAOC chemical structures, or by virtue of specific transport systems in the cell which recognize and transport the SAOC into the cell. In addition, the SAOC can be designed for administration only to certain selected cell populations by targeting the SAOC to be recognized by specific cellular uptake mechanisms which bind and take up the SAOC only within select cell populations.

For example, the SAOC may be designed to bind to a receptor found only in a certain cell
20 type, as discussed supra. The SAOC is also designed to recognize and selectively bind to target mRNA sequence, which may correspond to a sequence contained within the sequence shown in SEQ ID NO:1. The SAOC is designed to inactivate target mRNA sequence by either binding thereto and inducing degradation of the mRNA by, for example, RNase I digestion, or inhibiting translation of mRNA target sequence by interfering with the binding of translation-regulating factors or
25 ribosomes, or inclusion of other chemical structures, such as ribozyme sequences or reactive chemical groups which either degrade or chemically modify the target mRNA. SAOCs have been shown to be capable of such properties when directed against mRNA targets (see Cohen et al., TIPS, 10:435 (1989) and Weintraub, Sci. American, January (1990), p.40; both incorporated herein by reference).

30 In accordance with yet another embodiment of the present invention, there is provided a method for the recombinant production of the PTTG protein(s) of the invention by expressing the above-described nucleic acid sequences in suitable host cells. Recombinant DNA expression systems that are suitable to produce PTTG proteins described herein are well-known in the art. For example, the above-described nucleotide sequences can be incorporated into vectors for further
35 manipulation. As used herein, vector (or plasmid) refers to discrete elements that are used to introduce heterologous DNA into cells for either expression or replication thereof.

Suitable expression vectors are well-known in the art, and include vectors capable of expressing DNA operatively linked to a regulatory sequence, such as a promoter region that is capable of regulating expression of such DNA. Thus, an expression vector refers to a recombinant DNA or RNA construct, such as a plasmid, a phage, recombinant virus or other vector that, upon introduction into an appropriate host cell, results in expression of the inserted DNA. Appropriate expression vectors are well known to those of skill in the art and include those that are replicable in eukaryotic cells and/or prokaryotic cells and those that remain episomal or those which integrate into the host cell genome. In addition, vectors may contain appropriate packaging signals that enable the vector to be packaged by a number of viral virions, e.g., retroviruses, herpes viruses, adenoviruses, resulting in the formation of a "viral vector".

As used herein, a promoter region refers to a segment of DNA that controls transcription of DNA to which it is operatively linked. The promoter region includes specific sequences that are sufficient for RNA polymerase recognition, binding and transcription initiation. In addition, the promoter region includes sequences that modulate this recognition, binding and transcription initiation activity of RNA polymerase. These sequences may be cis acting or may be responsive to trans acting factors. Promoters, depending upon the nature of the regulation, may be constitutive or regulated. Exemplary promoters contemplated for use in the practice of the present invention include the SV40 early promoter, the cytomegalovirus (CMV) promoter, the mouse mammary tumor virus (MMTV) steroid-inducible promoter, Moloney murine leukemia virus (MMLV) promoter, and the like.

As used herein, the term "operatively linked" refers to the functional relationship of DNA with regulatory and effector nucleotide sequences, such as promoters, enhancers, transcriptional and translational stop sites, and other signal sequences. For example, operative linkage of DNA to a promoter refers to the physical and functional relationship between the DNA and the promoter such that the transcription of such DNA is initiated from the promoter by an RNA polymerase that specifically recognizes, binds to and transcribes the DNA.

As used herein, expression refers to the process by which polynucleic acids are transcribed into mRNA and translated into peptides, polypeptide, or proteins. If the polynucleic acid is derived from genomic DNA, expression may, if an appropriate eukaryotic host cell or organism is selected, include splicing of the mRNA.

Prokaryotic transformation vectors are well-known in the art and include pBlueskript and phage Lambda ZAP vectors (Stratagene, La Jolla, CA), and the like. Other suitable vectors and promoters are disclosed in detail in U.S. Patent No. 4,798,885, issued January 17, 1989, the disclosure of which is incorporated herein by reference in its entirety.

Other suitable vectors for transformation of E. coli cells include the pET expression vectors (Novagen, see US Patent 4,952,496), e.g., pET11a, which contains the T7 promoter, T7

terminator, the inducible *E. coli* lac operator, and the lac repressor gene; and pET 12a-c, which contain the T7 promoter, T7 terminator, and the *E. coli* ompT secretion signal. Another suitable vector is the pIN-IIIompA2 (see Duffaud et al., Meth. in Enzymology, 153:492-507, 1987), which contains the lpp promoter, the lacUV5 promoter operator, the ompA secretion signal, and the lac repressor gene.

Exemplary, eukaryotic transformation vectors, include the cloned bovine papilloma virus genome, the cloned genomes of the murine retroviruses, and eukaryotic cassettes, such as the pSV-2 gpt system (described by Mulligan and Berg, 1979, Nature Vol. 277:108-114) the Okayama-Berg cloning system (Mol. Cell Biol. Vol. 2:161-170, 1982), and the expression cloning vector described by Genetics Institute (Science Vol. 228:810-815, 1985), are available which provide substantial assurance of at least some expression of the protein of interest in the transformed eukaryotic cell line.

Particularly preferred base vectors which contain regulatory elements that can be linked to the invention PTTG-encoding DNAs for transfection of mammalian cells are cytomegalovirus (CMV) promoter-based vectors such as pcDNA1 (Invitrogen, San Diego, CA), MMTV promoter-based vectors such as pMAMNeo (Clontech, Palo Alto, CA) and pMSG (Pharmacia, Piscataway, NJ), and SV40 promoter-based vectors such as pSV β (Clontech, Palo Alto, CA).

In accordance with another embodiment of the present invention, there are provided "recombinant cells" containing the nucleic acid molecules (i.e., DNA or mRNA) of the present invention. Methods of transforming suitable host cells, preferably bacterial cells, and more preferably *E. coli* cells, as well as methods applicable for culturing said cells containing a gene encoding a heterologous protein, are generally known in the art. See, for example, Sambrook et al., Molecular Cloning: A Laboratory Manual (2 ed.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA (1989).

Exemplary methods of introducing (transducing) expression vectors containing invention nucleic acids into host cells to produce transduced recombinant cells (i.e., cells containing recombinant heterologous nucleic acid) are well-known in the art (see, for review, Friedmann, 1989, Science, 244:1275-1281; Mulligan, 1993, Science, 260:926-932, each of which are incorporated herein by reference in their entirety). Exemplary methods of transduction include, e.g., infection employing viral vectors (see, e.g., U.S. Patent 4,405,712 and 4,650,764), calcium phosphate transfection (U.S. Patents 4,399,216 and 4,634,665), dextran sulfate transfection, electroporation, lipofection (see, e.g., U.S. Patents 4,394,448 and 4,619,794), cytofection, particle bead bombardment, and the like. The heterologous nucleic acid can optionally include sequences which allow for its extrachromosomal (i.e., episomal) maintenance, or the heterologous DNA can be caused to integrate into the genome of the host (as an alternative means to ensure stable maintenance in the host).

Host organisms contemplated for use in the practice of the present invention include those organisms in which recombinant production of heterologous proteins has been carried out. Examples of such host organisms include bacteria (e.g., *E. coli*), yeast (e.g., *Saccharomyces cerevisiae*, *Candida tropicalis*, *Hansenula polymorpha* and *P. pastoris*; see, e.g., US Patent Nos. 4,882,279, 4,837,148, 4,929,555 and 4,855,231), mammalian cells (e.g., HEK293, CHO and Ltk cells), insect cells, and the like. Presently preferred host organisms are bacteria. The most preferred bacteria is *E. coli*.

In one embodiment, nucleic acids encoding the PTTG proteins of the invention may be delivered into mammalian cells, either in vivo or in vitro using suitable viral vectors well-known in the art, e.g., retroviral vectors, adenovirus vectors, and the like. In addition, where it is desirable to limit or reduce the in vivo expression of the PTTG of this invention, the introduction of the antisense strand of the invention nucleic acid is contemplated.

Viral based systems provide the advantage of being able to introduce relatively high levels of the heterologous nucleic acid into a variety of cells. Suitable viral vectors for introducing PTTG nucleic acid encoding a PTTG protein into mammalian cells (e.g., vascular tissue segments) are well known in the art. These viral vectors include, for example, Herpes simplex virus vectors (e.g., Geller et al., 1988, *Science*, 241:1667-1669), Vaccinia virus vectors (e.g., Piccini et al., 1987, *Meth. in Enzymology*, 153:545-563; Cytomegalovirus vectors (Mocarski et al., in *Viral Vectors*, Y. Gluzman and S.H. Hughes, Eds., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1988, pp. 78-84), Moloney murine leukemia virus vectors (Danos et al., 1980, *PNAS, USA*, 85:6469), adenovirus vectors (e.g., Logan et al., 1984, *PNAS, USA*, 81:3655-3659; Jones et al., 1979, *Cell*, 17:683-689; Berkner, 1988, *Biotechniques*, 6:616-626; Cotten et al., 1992, *PNAS, USA*, 89:6094-6098; Graham et al., 1991, *Meth. Mol. Biol.*, 7:109-127), adeno-associated virus vectors, retrovirus vectors, and the like. See, e.g., US Patents 4,405,712 and 4,650,764. Especially preferred viral vectors are the adenovirus and retroviral vectors.

For example, in one embodiment of the present invention, adenovirus-transferrin/polylysine-DNA (TfAdpl-DNA) vector complexes (Wagner et al., 1992, *PNAS, USA*, 89:6099-6103; Curiel et al., 1992, *Hum. Gene Therapy*, 3:147-154; Gao et al., 1993, *Hum. Gene Ther.*, 4:14-24) are employed to transduce mammalian cells with heterologous PTTG nucleic acid. Any of the plasmid expression vectors described herein may be employed in a TfAdpl-DNA complex.

As used herein, "retroviral vector" refers to the well-known gene transfer plasmids that have an expression cassette encoding an heterologous gene residing between two retroviral LTRs. Retroviral vectors typically contain appropriate packaging signals that enable the retroviral vector, or RNA transcribed using the retroviral vector as a template, to be packaged into a viral virion in an appropriate packaging cell line (see, e.g., U.S. Patent 4,650,764).

Suitable retroviral vectors for use herein are described, for example, in U.S. Patent 5,252,479, and in WIPO publications WO 92/07573, WO 90/06997, WO 89/05345, WO 92/05266 and WO 92/14829, incorporated herein by reference, which provide a description of methods for efficiently introducing nucleic acids into human cells using such retroviral vectors. Other retroviral vectors include, for example, the mouse mammary tumor virus vectors (e.g., Shackleford et al., 1988, PNAS, USA, 85:9655-9659), and the like.

In accordance with yet another embodiment of the present invention, there are provided anti-PTTG antibodies having specific reactivity with PTTG polypeptide of the present invention. Active fragments of antibodies are encompassed within the definition of "antibody". Invention antibodies can be produced by methods known in the art using the PTTG polypeptide of the invention, proteins or portions thereof as antigens. For example, polyclonal and monoclonal antibodies can be produced by methods well known in the art, as described, for example, in Harlow and Lane, *Antibodies: A Laboratory Manual* (Cold Spring Harbor Laboratory (1988)), which is incorporated herein by reference. The PTTG polypeptide of the invention may be utilized as immunogens in generating such antibodies. Alternatively, synthetic peptides can be prepared (using commercially available synthesizers) and used as immunogens. Amino acid sequences can be analyzed by methods well known in the art to determine whether they encode hydrophobic or hydrophilic domains of the corresponding polypeptide. Altered antibodies such as chimeric, humanized, CDR-grafted or bifunctional antibodies can also be produced by methods well known in the art. Such antibodies can also be produced by hybridoma, chemical synthesis or recombinant methods described, for example, in Sambrook et al., *supra.*, and Harlow and Lane, *supra.* Both anti-peptide and anti-fusion protein antibodies can be used. (see, for example, Bahouth et al., *Trends Pharmacol. Sci.* 12:338 (1991); Ausubel et al., *Current Protocols in Molecular Biology* (John Wiley and Sons, NY (1989) which are incorporated herein by reference).

Antibody so produced can be used, inter alia, in diagnostic methods and systems to detect the level of PTTG protein present in a mammalian, preferably human, body sample, such as tissue or vascular fluid. Such antibodies can also be used for the immunoaffinity or affinity chromatography purification of the PTTG protein of the invention. In addition, methods are contemplated herein for detecting the presence of PTTG polypeptide either on the surface of a cell or within a cell (such as within the nucleus), which methods comprise contacting the cell with an antibody that specifically binds to PTTG polypeptide, under conditions permitting binding of the antibody to PTTG polypeptide, detecting the presence of the antibody bound to PTTG, and thereby detecting the presence of invention polypeptide on the surface of, or within, the cell. With respect to the detection of such polypeptide, the antibodies can be used for in vitro diagnostic or in vivo imaging methods.

Immunological procedures useful for in vitro detection of target PTTG polypeptide in a

sample include immunoassays that employ a detectable antibody. Such immunoassays include, for example, ELISA, Pandex microfluorimetric assay, agglutination assays, flow cytometry, serum diagnostic assays and immunohistochemical staining procedures which are well known in the art. An antibody can be made detectable by various means well known in the art. For example, a
5 detectable marker can be directly or indirectly attached to the antibody. Useful markers include, for example, radionuclides, enzymes, fluorogens, chromogens and chemiluminescent labels.

The anti-PTTG antibodies of the invention modulate activity of the PTTG polypeptide in living animals, in humans, or in biological tissues or fluids isolated therefrom. Accordingly, compositions comprising a carrier and an amount of an antibody having specificity for PTTG
10 polypeptide effective to block naturally occurring ligands or other PTTG-binding proteins from binding to invention PTTG polypeptide are contemplated herein. For example, a monoclonal antibody directed to an epitope of PTTG polypeptide molecules present on the surface of a cell and having an amino acid sequence substantially the same as an amino acid sequence for a cell surface epitope of an PTTG polypeptide including the amino acid sequence shown in SEQ ID NO:2, SEQ.
15 ID No: 4, and fragments thereof, may be useful for this purpose.

The present invention further provides transgenic non-human mammals that are capable of expressing exogenous nucleic acids encoding PTTG polypeptide. As employed herein, the phrase "exogenous nucleic acid" refers to nucleic acid sequence which is not native to the host, or which is present in the host in other than its native environment (e.g., as part of a genetically engineered
20 DNA construct). In addition to naturally occurring levels of PTTG, the PTTG proteins of this invention may either be overexpressed, underexpressed, or expressed in an inactive mutated form, such as in the well-known knock-out transgenics, in transgenic mammals.

Also provided are transgenic non-human mammals capable of expressing nucleic acids encoding PTTG polypeptide so mutated as to be incapable of normal activity, i.e., do not express
25 native PTTG. The present invention also provides transgenic non-human mammals having a genome comprising antisense nucleic acids complementary to nucleic acids encoding PTTG polypeptide, placed so as to be transcribed into antisense mRNA complementary to mRNA encoding PTTG polypeptide, which hybridizes to the mRNA and, thereby, reduces the translation thereof. The nucleic acid may additionally comprise an inducible promoter and/or tissue specific regulatory
30 elements, so that expression can be induced, or restricted to specific cell types. Examples of nucleic acids are DNA or cDNA having a coding sequence substantially the same as the coding sequence shown in SEQ ID NO:1. An example of a non-human transgenic mammal is a transgenic mouse.

Animal model systems which elucidate the physiological and behavioral roles of PTTG polypeptide are also provided, and are produced by creating transgenic animals in which the
35 expression of the PTTG polypeptide is altered using a variety of techniques. Examples of such techniques include the insertion of normal or mutant versions of nucleic acids encoding an PTTG

polypeptide by microinjection, retroviral infection or other means well known to those skilled in the art, into appropriate fertilized embryos to produce a transgenic animal. (See, for example, Hogan et al., *Manipulating the Mouse Embryo: A Laboratory Manual* (Cold Spring Harbor Laboratory, (1986)).

5 Also contemplated herein, is the use of homologous recombination of mutant or normal versions of PTTG genes with the native gene locus in transgenic animals, to alter the regulation of expression or the structure of PTTG polypeptide (see, Capecchi et al., *Science* 244:1288 (1989); Zimmer et al., *Nature* 338:150 (1989); which are incorporated herein by reference). Homologous recombination techniques are well known in the art. Homologous recombination replaces the native
10 (endogenous) gene with a recombinant or mutated gene to produce an animal that cannot express native (endogenous) protein but can express, for example, a mutated protein which results in altered expression of PTTG polypeptide.

In contrast to homologous recombination, microinjection adds genes to the host genome, without removing host genes. Microinjection can produce a transgenic animal that is capable of
15 expressing both endogenous and exogenous PTTG protein. Inducible promoters can be linked to the coding region of nucleic acids to provide a means to regulate expression of the transgene. Tissue specific regulatory elements can be linked to the coding region to permit tissue-specific expression of the transgene. Transgenic animal model systems are useful for in vivo screening of compounds for identification of specific ligands, i.e., agonists and antagonists, which activate or
20 inhibit protein responses.

Invention nucleic acids, oligonucleotides (including antisense), vectors containing same, transformed host cells, polypeptide and combinations thereof, as well as antibodies of the present invention, can be used to screen compounds in vitro to determine whether a compound functions as a potential agonist or antagonist to the PTTG polypeptide of the invention. These in vitro
25 screening assays provide information regarding the function and activity of the PTTG polypeptide of the invention, which can lead to the identification and design of compounds that are capable of specific interaction with one or more types of polypeptide, peptides or proteins.

In accordance with still another embodiment of the present invention, there is provided a method for identifying compounds which bind to PTTG polypeptide. The invention proteins may
30 be employed in a competitive binding assay. Such an assay can accommodate the rapid screening of a large number of compounds to determine which compounds, if any, are capable of binding to PTTG proteins. Subsequently, more detailed assays can be carried out with those compounds found to bind, to further determine whether such compounds act as modulators, agonists or antagonists of invention proteins.

35 In another embodiment of the invention, there is provided a bioassay for identifying compounds which modulate the activity of the PTTG polypeptide of the invention. According to

5 this method, the PTTG polypeptides of the invention are contacted with an "unknown" or test substance (in the presence of a reporter gene construct when antagonist activity is tested), the activity of the polypeptide is monitored subsequent to the contact with the "unknown" or test substance, and those substances which cause the reporter gene construct to be expressed are identified as functional ligands for PTTG polypeptide.

10 In accordance with another embodiment of the present invention, transformed host cells that recombinantly express the PTSG polypeptide of the invention may be contacted with a test compound, and the modulating effect(s) thereof can then be evaluated by comparing the PTTG-mediated response (e.g., via reporter gene expression) in the presence and absence of test compound, or by comparing the response of test cells or control cells (i.e., cells that do not express PTTG polypeptide), to the presence of the compound.

15 As used herein, a compound or a signal that "modulates the activity" of PTTG polypeptide of this invention refers to a compound or a signal that alters the activity of PTTG polypeptide so that the activity of the invention PTTG polypeptide is different in the presence of the compound or signal than in the absence of the compound or signal. In particular, such compounds or signals include agonists and antagonists. An agonist encompasses a compound or a signal that activates PTTG protein function. Alternatively, an antagonist includes a compound or signal that interferes with PTTG protein function. Typically, the effect of an antagonist is observed as a blocking of agonist-induced protein activation. Antagonists include competitive and non-competitive antagonists. A competitive antagonist (or competitive blocker) interacts with or near the site specific for agonist binding. A non-competitive antagonist or blocker inactivates the function of the polypeptide by interacting with a site other than the agonist interaction site.

20 As understood by those of skill in the art, assay methods for identifying compounds that modulate PTTG activity generally require comparison to a control. One type of a "control" is a cell or culture that is treated substantially the same as the test cell or test culture exposed to the compound, with the distinction that the "control" cell or culture is not exposed to the compound. For example, in methods that use voltage clamp electrophysiological procedures, the same cell can be tested in the presence or absence of compound, by merely changing the external solution bathing the cell. Another type of "control" cell or culture may be a cell or culture that is identical to the transfected cells, with the exception that the "control" cell or culture do not express native proteins. Accordingly, the response of the transfected cell to compound is compared to the response (or lack thereof) of the "control" cell or culture to the same compound under the same reaction conditions.

30 In yet another embodiment of the present invention, the activation of PTTG polypeptide can be modulated by contacting the polypeptide with an effective amount of at least one compound identified by the above-described bioassays.

35 In accordance with another embodiment of the present invention, there are provided

methods for diagnosing or detecting a pathological mass (such as, for example, an endocrine or non-endocrine tumor, atherosclerotic plaque, and the like), said method comprising detecting, in cells of a subject, a transcribed or mutant sequence including SEQ ID NO:1.

In a particular embodiment, the invention diagnostic methods described herein are useful for differential diagnosis of malignant versus benign tumors in biopsy specimens, and the like. In another embodiment, the invention diagnostic methods described herein are useful for predicting tumor behavior and responsiveness to therapy.

In accordance with another embodiment of the present invention, there are provided methods for diagnosing pituitary tumors, said method comprising detecting, in pituitary-derived cells of a subject, a transcribed mRNA sequence including SEQ ID NO:1.

In accordance with another embodiment of the present invention, there are provided diagnostic systems, preferably in kit form, comprising at least one invention nucleic acid in a suitable packaging material. The diagnostic nucleic acids are derived from the PTTG-encoding nucleic acids described herein. In one embodiment, for example, the diagnostic nucleic acids are derived from SEQ ID NO:1. Invention diagnostic systems are useful for assaying for the presence or absence of nucleic acid encoding PTTG in either genomic DNA or in transcribed nucleic acid (such as mRNA or cDNA) encoding PTTG in any tumors (e.g., pituitary, and the like) or diseased tissue. Invention diagnostic systems contemplated herein, may make use of well-known polymerase chain reaction (PCR) or RTPCR (reverse-transcriptase-PCR) methodologies.

A suitable diagnostic system includes at least one invention nucleic acid, preferably two or more invention nucleic acids, as a separately packaged chemical reagent(s) in an amount sufficient for at least one assay. Instructions for use of the packaged reagent are also typically included. Those of skill in the art can readily incorporate invention nucleic probes and/or primers into kit form in combination with appropriate buffers and solutions for the practice of the invention methods as described herein.

As employed herein, the phrase "packaging material" refers to one or more physical structures used to house the contents of the kit, such as invention nucleic acid probes or primers, and the like. The packaging material is constructed by well known methods, preferably to provide a sterile, contaminant-free environment. The packaging material has a label which indicates that the invention nucleic acids can be used for detecting a particular sequence encoding PTTG including the nucleotide sequence set forth in SEQ ID NO:1 or a mutant thereof, thereby diagnosing the presence of, or a predisposition for, a particular pathology (such as, for example, pituitary tumorigenesis, and the like). In addition, the packaging material contains instructions indicating how the materials within the kit are employed both to detect a particular sequence and diagnose the presence of, or a predisposition for, a particular pathology.

The packaging materials employed herein in relation to diagnostic systems are those

customarily utilized in nucleic acid-based diagnostic systems. As used herein, the term "package" refers to a solid matrix or material such as glass, plastic, paper, foil, and the like, capable of holding within fixed limits an isolated nucleic acid, oligonucleotide, or primer of the present invention. Thus, for example, a package can be a glass vial used to contain milligram quantities of a contemplated nucleic acid, oligonucleotide or primer, or it can be a microtiter plate well to which microgram quantities of a contemplated nucleic acid probe have been operatively affixed.

"Instructions for use" typically include a tangible expression describing the reagent concentration or at least one assay method parameter, such as the relative amounts of reagent and sample to be admixed, maintenance time periods for reagent/sample admixtures, temperature, buffer conditions, and the like.

All US patents and all publications mentioned herein are incorporated in their entirety by reference thereto. The invention will now be described in greater detail by reference to the following non-limiting examples.

Unless otherwise stated, the present invention was performed using standard procedures, as described, for example in Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA (1982); Sambrook et al., *Molecular Cloning: A Laboratory Manual* (2 ed.), Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA (1989); Davis et al., *Basic Methods in Molecular Biology*, Elsevier Science Publishing, Inc., New York, USA (1986); or *Methods in Enzymology: Guide to Molecular Cloning Techniques Vol.152*, S. L. Berger and A. R. Kimmerl Eds., Academic Press Inc., San Diego, USA (1987).

EXAMPLES

Example 1: Isolation of PTTG cDNA

To clarify the molecular mechanisms involved in pituitary tumorigenesis, differential display PCR was used to identify mRNAs differentially expressed in pituitary tumor cells (see, e.g., Risinger et al., 1994, *Molec. Carcinogenesis*, 11:13-18; and Qu et al., 1996, *Nature*, 380:243-247). GC and GH₄ pituitary tumor cell lines (ATCC #CCL-82 and #CCL-82.1, respectively) and an osteogenic sarcoma cell line UM108 (ATCC #CRL-1663) were grown in DMEM supplemented with 10% fetal bovine serum. Normal Sprague-Dawley rat pituitaries were freshly excised. Total RNA was extracted from tissue cultured cells and pituitary tissue using RNeasy™ kit (Qiagen) according to manufacturer's instructions. Trace DNA contamination in RNA preparations was removed by DNaseI (GenHunter Corporation) digestion. cDNA was synthesized from 200 ng total RNA using MMLV reverse transcriptase (GenHunter Corporation), and one of the three anchored primers (GenHunter Corporation). The cDNA generated was used in the PCR display.

Three downstream anchored primers AAGCT₁₁X (where X may be A, G, or C), were used

in conjunction with 40 upstream arbitrary primers for PCR display. 120 primer pairs were used to screen mRNA expression in pituitary tumors versus normal pituitary. One tenth of the cDNA generated from the reverse transcriptase reaction was amplified using AmpliTaq DNA polymerase (Perkin Elmer) in a total volume of 20 μ l containing 10 mM Tris, pH 8.4, 50 mM KCl, 1.5 mM MgCl_2 , 0.001% gelatin, 2 μ M dNTPs, 0.2 μ M each primer and 1 μ l [^{35}S]dATP. PCR cycles consisted of 30 seconds at 94°C, 2 minutes at 40°C, and 30 seconds at 72°C for 40 cycles. The products were separated on 6% sequencing gels, and dried gels were exposed to Kodak film for 24 to 48 hours.

After development, DNA fragments amplified from pituitary tumor and normal pituitary were compared. Bands unique to pituitary tumor were excised from the gel, and DNA extracted by boiling in 100 μ l water and precipitated with ethanol in the presence of glycogen (GenHunter Corporation). DNA was reamplified using the original set of primers and the same thermal cycling conditions except that the dNTP concentration was increased to 20 μ M. Reaction products were run on 1% agarose gel and stained with ethidium bromide. Bands were excised from the gel, eluted (Qiagen), cloned in to TA vectors (Invitrogen) and sequenced using sequenase (USB). Using 120 primer pairs in the above-described PCR assay, 11 DNA bands that appeared to be differentially expressed in pituitary tumor cells were identified. These bands were evaluated further by Northern blot analysis, using the PCR products as probes.

For Northern blot analysis, 20 μ g of total RNA were fractionated on 1% agarose gel, blotted on to nylon membrane and hybridized with random primed probe using Quickhyb solutions (Stratagene). After washing, membranes were exposed to Kodak films for 6 to 72 hours. As a result of the Northern blot assay, pituitary tumor specific signals were detected for 2 bands. DNA sequence analysis revealed that one sequence was homologous with Insulin-induced growth response protein, while the another 396 base pair fragment (amplified using 5' AAGCTTTTTTTTTTTTG 3' as the anchored primer and 5' AAGCTTGCTGCTC 3' as an arbitrary primer) showed no homology to known sequences in the GenBank. This 396 bp fragment detected a highly expressed mRNA of about 1.3 kb in pituitary tumor cells, but not in normal pituitary nor in osteogenic sarcoma cells.

Example 2: Characterization of PTTG cDNA

To characterize this pituitary tumor-specific mRNA further, a cDNA library was constructed using mRNA isolated from rat pituitary tumor cells. Poly A⁺RNA was isolated from pituitary tumor GH₄ cells using messenger RNA isolation kit (Stratagene) according to manufacturer's instructions, and was used to construct a cDNA library in ZAP Express vectors (Stratagene). The cDNA library was constructed using ZAP ExpressTM cDNA synthesis and Gigapack III gold cloning kit (Stratagene) following manufacturer's instructions. The library was screened using the 396 bp differentially displayed PCR product (cloned into TA vector) as the

probe. After tertiary screening, positive clones were excised by *in vivo* excision using helper phage. The resulting pBK-CMV phagemid containing the insert was identified by Southern Blotting analysis. Unidirectional nested deletions were made into the DNA insert using EXOIII/Mung bean nuclease deletion kit (Stratagene) following manufacturer's instructions. Both strands of the insert
5 DNA were sequenced using Sequenase (USB).

Using the 396 bp PCR fragment described in Example 1 as a probe, a cDNA clone of 974 bp was isolated and characterized. This cDNA was designated as pituitary-tumor-transforming gene (PTTG). The sequence of PTTG contains an open reading frame for 199 amino acids (SEQ ID NO:2). The presence of an in-frame stop codon upstream of the predicted initiation codon indicates
10 that PTTG contains the complete ORF. The nucleic acid and protein sequences are provided in Table 1 below.

Table 1: PTTG Nucleic Acid and Protein Sequences

5	AATTCGGGCAC	GAGCCAACCT	TGAGCATCTG	ATCCTCTTGG	CTTCTCCTTC	CTATCGCTGA	60
	GCTGGTAGGC	TGGAGACAGT	TGTTTGGGTG	CCAACATCAA	CAAACGATTT	CTGTAGTTTA	120
	GCGTTTATGA	CCCTGGCGTG	AAGATTTAAG	GTCTGGATTA	AGCCTGTTGA	CTTCTCCAGC	180
	TACTTCTAAA	TTTTTGTGCA	TAGGTGCTCT	GGTCTCTGTT	GCTGCTTAGT	TCTTCCAGCC	240
	TTCCTCAATG	CCAGTTTTAT	AATATGCAGG	TCTCTCCCCT	CAGTAATCCA	GG ATG	295
					Met		
					1		
10	GCT ACT CTG ATC TTT GTT GAT AAG GAT AAC GAA GAG CCA GGC AGC CGT						343
	Ala Thr Leu Ile Phe Val Asp Lys Asp Asn Glu Glu Pro Gly Ser Arg						
		5	10	15			
	TTG GCA TCT AAG GAT GGA TTG AAG CTG GGC TCT GGT GTC AAA GCC TTA						391
	Leu Ala Ser Lys Asp Gly Leu Lys Leu Gly Ser Gly Val Lys Ala Leu						
15		20	25	30			
	GAT GGG AAA TTG CAG GTT TCA ACG CCA CGA GTC GGC AAA GTG TTC GGT						439
	Asp Gly Lys Leu Gln Val Ser Thr Pro Arg Val Gly Lys Val Phe Gly						
		35	40	45			
20	GCC CCA GGC TTG CCT AAA GCC AGC AGG AAG GGT CTG GGA ACT GTC AAC						487
	Ala Pro Gly Leu Pro Lys Ala Ser Arg Lys Ala Leu Gly Thr Val Asn						
		50	55	60	65		
	AGA GTT ACT GAA AAG CCA GTG AAG AGT AGT AAA CCC CTG CAA TCG AAA						535
	Arg Val Thr Glu Lys Pro Val Lys Ser Ser Lys Pro Leu Gln Ser Lys						
		70	75	80			
25	CAG CCG ACT CTG AGT GTG AAA AAG ATC ACC GAG AAG TCT ACT AAG ACA						583
	Gln Pro Thr Leu Ser Val Lys Lys Ile Thr Glu Lys Ser Thr Lys Thr						
		85	90	95			
	CAA GGC TCT GCT CCT GCT CCT GAT GAT GCC TAC CCA GAA ATA GAA AAG						631
	Gln Gly Ser Ala Pro Ala Pro Asp Asp Ala Tyr Pro Glu Ile Glu Lys						
30		100	105	110			
	TTC TTC CCC TTC GAT CCT CTA GAT TTT GAG AGT TTT GAC CTG CCT GAA						679
	Phe Phe Pro Phe Asp Pro Leu Asp Phe Glu Ser Phe Asp Leu Pro Glu						
		115	120	125			
	GAG CAC CAG ATC TCA CTT CTC CCC TTG AAT GGA GTG CCT CTC ATG ATC						727
35	Glu His Gln Ile Ser Leu Leu Pro Leu Asn Gly Val Pro Leu Met Ile						
		130	135	140	145		
	CTG AAT GAA GAG AGG GGG CTT GAG AAG CTG CTG CAC CTG GAC CCC CCT						775
	Leu Asn Glu Glu Arg Gly Leu Glu Lys Leu Leu His Leu Asp Pro Pro						
		150	155	160			
40	TCC CCT CTG CAG AAG CCC TTC CTA CCG TGG GAA TCT GAT CCG TTG CCG						823
	Ser Pro Leu Gln Lys Pro Phe Leu Pro Trp Glu Ser Asp Pro Leu Pro						
		165	170	175			
	TCT CCT CCC AGC GCC CTC TCC GCT CTG GAT GTT GAA TTG CCG CCT GTT						871
	Ser Pro Pro Ser Ala Leu Ser Ala Leu Asp Val Glu Leu Pro Pro Val						
45		180	185	190			
	TGT TAC GAT GCA GAT ATT TAAACGTCTT ACTCCTTTAT AGTTTATGTA						919
	Cys Tyr Asp Ala Asp Ile						
		195	200				
50	AGTTGTATTA ATAAAGCATT TGTGTGTAAA AAAAAAAAAA AAAACTCGAG AGTAC						974

This was verified by demonstrating both in vitro transcription and in vitro translation of the gene product as described in Example 3.

Example 3: In vitro Transcription & Translation of PTTG

Sense and antisense PTTG mRNAs were *in vitro* transcribed using T3 and T7 RNA polymerase (Stratagene), respectively. The excess template was removed by DNase I digestion. The *in vitro* transcribed mRNA was translated in rabbit reticular lysate (Stratagene). Reactions were carried out at 30°C for 60 minutes, in a total volume of 25 μ l containing 3 μ l *in vitro* transcribed RNA, 2 μ l 35 S-Methionine (Dupond) and 20 μ l lysate. Translation products were analyzed by SDS-PAGE (15% resolving gel and 5% stacking gel), and exposed to Kodak film for 16 hours.

The results indicate that translation of *in vitro* transcribed PTTG sense mRNA results in a protein of approximately 25 KD on SDS-PAGE, whereas no protein was generated in either the reaction without added mRNA or when PTTG antisense mRNA was utilized.

Example 4: Expression of PTTG mRNA

A search of GenBank and a protein profile analysis (using a BLAST Program search of databases of the national center for Biotechnology Information) indicated that PTTG shares no homology with known sequences, and its encoded protein is highly hydrophilic, and contains no well recognized functional motifs. The tissue expression pattern of PTTG mRNA was studied by Northern Blot analysis. A rat multiple tissue Northern blot was purchased from Clontech. Approximately 2 μ g of poly A+ RNA per lane from eight different rat tissues (heart, brain, spleen, lung, liver, skeletal muscle, kidney, and testis) was run on a denaturing formaldehyde 1.2% agarose gel, transferred to nylon membrane and UV-cross linked. The membrane was first hybridized to the full length PTTG cDNA probe, and was stripped and rehybridized to a human β -actin cDNA control probe. Hybridization was performed at 60°C for one hour in ExpressHyb hybridization solution (Clontech). Washing was twice 15 minutes at room temperature in 2xSSC, 0.05% SDS, and twice 15 minutes at 50°C in 0.1% SSC, 0.1% SDS. Exposure time for PTTG probe was 24 hrs, and actin probe 2 hours.

The results of the Northern assay indicate that testis is the only tissue, other than pituitary tumor cells, that expresses PTTG mRNA, and the testis expression level is much lower (2 μ g polyA+ mRNA, 24 hour exposure) than in pituitary tumor cells (20 μ g total RNA, 6 hour exposure). Interestingly, the testicular transcript (about 1 Kb) is shorter than the transcript in pituitary tumors (1.3 Kb), indicating that the mRNA is differentially spliced in testis, and that the 1.3 Kb transcript is specific for pituitary tumor cells.

Example 5: Over-expression of PTTG by NIH 3T3 Fibroblast Cells

Since PTTG mRNA is over-expressed in pituitary tumor cells, whether this protein exerts an effect on cell proliferation and transformation was determined. An eukaryotic expression vector containing the entire coding region of PTTG was stably transfected into NIH 3T3 fibroblasts.

The entire coding region of the PTTG was cloned in frame into pBK-CMV eukaryotic expression vector (Stratagene), and transfected into NIH 3T3 cells by calcium precipitation. 48 hrs after transfection, cells were diluted 1:10 and grown in selection medium containing 1 mg/ml G418 for two weeks in when individual clones were isolated. Cell extracts were prepared from each colony and separated on 15% SDS-polyacrylamide gels, and blotted onto nylon membrane. A polyclonal antibody was generated using the first 17 amino acids of PTTG as epitope (Research Genetics). The antibody was diluted 1:5000 and incubated with the above membrane at room temperature for 1 hour. After washing, the membrane was incubated with horseradish peroxidase-labeled secondary antibody for one hour at room temperature. The hybridization signal was detected by enhanced chemiluminescence (ECL detection system, Amersham).

Expression levels of the PTTG were monitored by immunoblot analysis using the above-described specific polyclonal antibody directed against the first 17 amino acids of the protein. Expression levels of individual clones varied, and clones that expressed higher protein levels were used for further analysis.

Example 6: Effect of PTTG Expression on Cell Proliferation

A nonradioactive cell proliferation assay was used to determine the effect of PTTG protein over-expression on cell proliferation (see, e.g., Mosmann, T., 1983, J. Immunol. Meth., 65:55-63; and Carmichael et al., 1987, Cancer Res., 47:943-946). Cell proliferation was assayed using CellTiter 96TM Non-radioactive cell proliferation Assay kit (Promega) according to the manufacturer's instructions. Five thousand cells were seeded in 96 well plates (6 wells for each clone in each assay), and incubated at 37°C for 24 to 72 hours. At each time point, 15 µl of the Dye solution were added to each well, and incubated at 37°C for 4 hours. One hundred µl of the solubilization/stop solution were then added to each well. After one hour incubation, the contents of the wells were mixed, and absorbance at 595 nm was recorded using an ELISA reader. Absorbance at 595 nm correlates directly with the number of cells in each well.

Three independent experiments were performed and the results are shown in Figure 1. In Figure 1, the cell growth rate is expressed as absorbance at 595 nM. The error bars represent SEM (n=6). The results (Figure 1) show that the growth rate of 3T3 cells expressing PTTG protein (assayed by cellular conversion of tetrazolium into formazan) was suppressed 25 to 50% as compared with 3T3 cells expressing the pCMV vector alone, indicating that PTTG protein inhibits cell proliferation.

Example 7: PTTG Induction of Morphological Transformation and Soft-agar Growth of NIH 3T3 Cells

The transforming property of PTTG protein was demonstrated by its ability to form foci in

monolayer cultures and show anchorage-independent growth in soft agar, as shown in Table 2 below.

Table 2: Colony Formation by NIH 3T3 Cells Transfected with PTTG cDNA Constructs

5

Cell line	Growth in Soft Agar	Efficiency of Colony formation in Soft Agar (%)
No DNA	0	0
Vector only	1.3±0.7	0.013
PTTG 3	26±4.6	0.26
PTTG 4	132±26	1.32
PTTG 8	33±6.0	0.33
15 PTTG 9	72±13	0.72
PTTG 10	92±18	0.92

15

20

10⁴ cells/dish were plated as described in FIG. 7.
Plates were scored at 14 days.
Only colonies consist of at least 40 cells were counted.
Values are means ± SEM of triplicates.
Efficiency of colony formation was calculated as percentage of number of colonies divided by total number of cells.

25

30

As primary pituitary cells are an admixture of multiple cell types and they do not replicate in vitro, NIH 3T3 cells were employed. For the soft agar assay, 60 mm tissue culture plates were coated with 5 ml soft-agar (20% 2XDMEM, 50% DMEM, 10% fetal bovine serum, 20% 2.5% agar, melted and combined at 45°C). See, Schwab et al., 1985, Nature, 316:160-162. 2 ml cells suspended in medium were then combined with 4 ml agar mixture, and 1.5 ml of this mixture added to each plate. The cells were plated at a density of 10⁴ cells/dish and incubated for 14 days before counting the number of colonies and photography.

35

The results indicate that NIH 3T3 parental cells and 3T3 cells transfected with pCMV vector do not form colonies on soft agar, whereas 3T3 cells transfected with PTTG form large colonies. In addition, focal transformation is observed in cells over-expressing PTTG protein, but cells expressing pCMV vector without the PTTG insert showed similar morphology to the parental 3T3 cells.

Example 8: Determination of In Vivo PTTG Tumorigenicity

40

To determine whether PTTG is tumorigenic in vivo, PTTG-transfected 3T3 cells were injected subcutaneously into athymic nude mice. 3X10⁵ cells of either PTTG or pCMV vector alone transfected cells were resuspended in PBS and injected subcutaneously into nude mice (5 for each group). Tumors were excised from animals at the end of the 3rd week and weighted. All injected

animals developed large tumors (1-3 grams) within 3 weeks. The results are shown in Table 3 below. No mouse injected with vector only transfected cells developed tumors.

Table 3: In vivo Tumorigenesis by NIH 3T3 Cells Transfected with PTTG cDNA Expression Vector

Cell line	Animals injected	Tumor formation
Vector only	5	0/5
PTTG 4	5	5/5

3 x 10⁵ cells were injected subcutaneously into athymic nude mice. Tumors developed within 3 weeks and weighed 1-3 grams at sacrifice.

These results clearly indicate that PTTG is a potent transforming gene in vivo.

Example 9: Human Carcinoma Cell Lines Express PTTG

The expression of PTTG in various human cell lines was studied employing a multiple human cancer cell line Northern blot (Clontech). The specific cell lines tested are shown in Table 4 below.

Table 4: Human Carcinoma Cell Lines Tested

Cell Line	PTTG Expression
1 Promyelocytic Leukemia HL-60	+
2 HeLa Cell S3	+
3 Chronic Myelogenous Leukemia K-562	+
4 Lymphoblastic Leukemia MOLT-4	+
5 Burkitt's lymphoma Raji	+
6 Colorectal Adenocarcinoma SW 480	+
7 Lung Carcinoma A549	+
8 Melanoma G361	+

About 2 µg polyA RNA from each of the 8 cell lines indicated in Table 1 above were placed on each lane of a denaturing formaldehyde 1.2% agarose gel, separated by denaturing gel electrophoresis to ensure intactness, transferred to a charge-modified nylon membrane by Northern blotting, and fixed by UV irradiation. Lanes 1 to 8 contained RNA from promyelocytic leukemia HL-60, HeLa cell line S3, human chronic myelogenous leukemia K-562, lymphoblastic leukemia MOLT-4, Burkitt's lymphoma Raji, colorectal adenocarcinoma SW 480, lung carcinoma A549 and melanoma G361, respectively. RNA size marker lines at 9.5, 7.5, 4.4, 2.4, and 1.35 kb were

indicated in ink on the left margin of the blot, and utilized as sizing standards, and a notch was cut out from the lower left hand corner of the membrane to provide orientation. Radiolabeled human β -actin cDNA was utilized as a control probe for matching of different batches of polyA RNAs. A single control band at 2.0 kb in all lanes spotted is confirmatory.

5 The blots were probed with the full length rat PTTG cDNA probe (SEQ. ID No: 1; 974 bp) at 60°C for 1 hr. in ExpressHyb hybridization solution (Clontech) as described by Sambrook et al., the relevant section of which reference is incorporated herein by reference. See, Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd. Ed, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989). The blots were then washed twice for 15 min at room temperature in 10 2xSSC, 0.05% SDS, and twice for 15 min at 50°C in 0.1% SSC, 0.1% SDS. A more detailed description of the remaining experimental procedures may be found in Pei & Melmed, the relevant section of which is incorporated herein by reference. See, Pei & Melmed, Endocrinology 4: 433-441 (1997).

15 All cells tested by the Northern blot analysis as described above evidenced expression of human PTTG, including lymphoma, leukemia, melanoma and lung carcinomas, among others.

Example 10: Cloning of Human PTTG cDNA

20 A human fetal liver cDNA library (Clontech, Palo Alto, CA) was screened as described by Maniatis et al. using a radioactively labeled cDNA fragment of the entire rat PTTG coding region as a probe. See, Maniatis et al, Molecular cloning, Cold Spring Harbor Press, 1989. The cDNA inserts from positive clones were subcloned into plasmid pBluescript-SK (Stratagene, La Jolla, CA), and subjected to sequence analysis using Sequenase kit (U.S. Biochemical Corp., Cleveland, OH). The sequence of the nucleic acid is provided in Table 5 below.

Table 5: PTTG Nucleic Acid Sequences

	1	ATGGCCGCGA GTTGTGGTTT AAACCAGGAG TGCCGCGCGT CCGTTCACCG
	51	CGGCCTCAGA TGAATGCGGC TGTAAAGACC TGCAATAATC CAGAATG GCT
5	101	<u>ACT CTG ATC TAT GTT GAT AAG GAA AAT GGA GAA CCA GGC ACC</u>
	123	<u>CGT GTG GTT GCT AAG GAT GGG CTG AAG CTG GGG TCT GGA CCT</u>
	185	<u>TCA ATC AAA GCC TTA GAT GGG AGA TCT CAA GTT TCA ACA CCA</u>
	207	<u>CGT TTT GGC AAA ACG TTC GAT GCC CCA CCA GCC TTA CCT AAA</u>
	249	<u>GCT ACT AGA AAG GCT TTG GGA ACT GTC AAC AGA GCT ACA GAA</u>
10	311	<u>AAG TCT GTA AAG ACC AAG GGA CCC CTC AAA CAA AAA CAG CCA</u>
	350	<u>CCA AGC TTT TCT GCC AAA AAG ATG ACT GAG AAG ACT GTT AAA</u>
	392	<u>GCA AAA AGC TCT GTT CCT GCC TCA GAT GAT GCC TAT CCA GAA</u>
	334	<u>ATA GAA AAA TTC TTT CCC TTC AAT CCT CTA GAC TTT GAG AGT</u>
15	376	<u>TTT GAC CTG CCT GAA GAG CAC CAG ATT GCG CAC CTC CCC TTG</u>
	419	<u>AGT GGA GTG CCT CTC ATG ATC CTT GAC GAG GAG AGA GAG CTT</u>
	560	<u>GAA AAG CTG TTT CAG CTG GGC CCC CCT TCA CCT GTG AAG ATG</u>
	602	<u>CCC TCT CCA CCA TGG GAA TCC AAT CTG TTG CAG TCT CCT TCA</u>
	644	<u>AGC ATT CTG TCG ACC CTG GAT GTT GAA TTG CCA CCT GTT TGC</u>
	686	<u>TGT GAC ATA GAT ATT TAA</u>
20	704	ATTTCTT AGTGCTTCAG AGTTTGTGTG TATTTGTATT AATAAAGCAT
	751	TCTTTAACAG ATAAAAAAAA AAAAAAAAA (SEQ. ID No:3)

The open reading frame of 606 bp is indicated by underlining.

- 25 A complete open reading frame containing 606 bp was found in the positive clones. The homology between the nucleotide sequences of the open reading frame and the coding region of rat PTTG or PTTG (old nomenclature) is 85%. The deduced amino acid sequence is shown in Table 6 below.

Table 6: PTTG Amino Acid Sequence

30	1	MATLIYVDKE NGEPGTRVVA KDGLKLGSGP SIKALDGRSQ VSTPRFGKTF
	51	DAPPALPKAT RKALGTVNRA TEKSVKTKGP LKQKQPSFSA KKMTEKTVKA
	101	KSSVPASDDA YPEIEKFFPF NPLDFESFDL PEEHQIAHLP LSGVPLMILD
	151	EERELEKLFQ LGPPSPVKMP SPPWESNLLQ SPSSILSTLD VELPPVCCDI
35	201	DI*-

- 40 A comparison of the amino acid sequence the human PTTG translated product of this open reading frame and that of the rat PTTG protein reveals 77% identity and 89% homology. The cDNAs obtained from these clones represents human homologies of rat PTTG. No other cDNA fragments with higher homology were detected from the library.

Example 11: Tissue Distribution of Human PTTG mRNA

Total RNA was prepared using Trizol Reagent (Gibco-BRL, Gaithersburg, MD) from normal human pituitary glands (Zoion Research Inc. Worcester, MA) and fresh human pituitary tumors collected at surgery and frozen in liquid nitrogen. 20 mg total RNA were used for 1% agarose gel electrophoresis. RNA blots (Clontech, Palo Alto, CA) derived from normal adult and fetal tissues as well as from malignant tumor cell lines, were hybridized with radioactively labeled human cDNA fragment containing the complete coding region. The RNA isolated from each cell line was transferred onto a nylon membrane (Amersham, Arlington Heights, IL), and hybridized with radioactively labeled probe at 55°C overnight in 6xSSC, 2xDenhardt's solution, 0.25% SDS. The membranes were washed twice at room temperature for 15 minutes each, and then for 20 minutes at 60°C in 0.5xSSC, 0.1% SDS, and autoradiographed. The autoradiography was carried out using Kodak BIOMEX-MR film (Eastman Kodak, Rochester, NY) with an intensifying screen. The blots were stripped by washing for 20 minutes in distilled water at 95°C for subsequent probing.

The results from the Northern blot analysis indicated that PTTG is expressed in liver, but not in brain, lung, and kidney of human fetal tissue. In addition, PTTG is strongly expressed in testis, modestly expressed in thymus, and weakly expressed in colon and small intestine of normal human adult tissue. No expression was detected by Northern analysis in brain, heart, liver, lung, muscle, ovary, placenta, kidney, and pancreas.

The expression of PTTG in several human carcinoma cell lines was also analyzed by Northern blots. In every carcinoma cells examined, PTTG was found highly expressed. The human tumor cell lines tested are listed in Table 7 below.

Table 7: Tested Human Tumor Cell Lines

25	Promyelocytic leukemia HL-60
	Epitheloid carcinoma HeLa cell S3
	Chronic myelogenous leukemia K-562
	Lymphoblastic leukemia MOLT-4
	Burkitt's lymphoma Raji
30	Colorectal adenocarcinoma SW 480
	Lung carcinoma A549
	Melanoma G361
	Hepatocellular carcinoma Hep 3B
	Thyroid carcinoma TC-1
	Breast adenocarcinoma MCF-7
35	Osteogenic sarcoma U2 OS
	Placenta choriocarcinoma JAR
	Choriocarcinoma JEG-3

Example 12: Human PTTG expression in normal pituitary and pituitary tumors

RT-PCR was performed as follows. 5 µg total RNA were treated with 100 U RNase-free DNase I at room temperature for 15 minutes. DNase I was inactivated by incubation at 65° C for 15 minutes. The sample was then used for reverse transcription using oligo-dT primer and SuperScript II reverse transcriptase (Gibco-BRL, Gaithersburg, MD). After reverse transcription, the sample was subjected to PCR amplification with PCR SuperMix (Gibco-BRL, Gaithersburg, MD) using hPTTG-specific primers and human cyclophilin A-specific primers as an internal control.

Northern blot analysis indicated that the level of expression of PTTG is quite low in normal pituitary as well as in pituitary tumors. Therefore, comparative RT-PCR was used to study the expression of PTTG quantitatively in normal pituitary and pituitary tumors. The results of this study showed that in most of pituitary tumors tested, including non-functioning tumors, GH-secreting tumors, and prolactinomas, the expression level of PTTG was higher than that of normal pituitary.

Example 13: Stable Transfection of Human PTTG into NIH 3T3 Cells

The complete coding region of hPTTG cDNA was subcloned in reading frame into the mammalian expression vector pBK-CMV (Stratagene, La Jolla, CA), and transfected into NIH 3T3 fibroblast cells by Lipofectamine (Gibco-BRL, Gaithersburg, MD) according to manufacturer's protocol. 24 hours after transfection, the cells were serially diluted and grown in selection medium containing 1 mg/ml G418 for 2 weeks. Individual clones were isolated and maintained in selection medium. Total RNA was isolated from hPTTG-transfected cell lines as well as from control cells in which blank vector pBK-CMV had been transfected. Northern blot was performed to confirm overexpression of hPTTG in transfected cell lines. These cell lines were used in subsequent cell proliferation assay as well as in vitro and in vivo transformation assay.

Example 14: Cell Proliferation Assay

A cell proliferation assay was performed using the CellTiter 96 non-radioactive cell proliferation assay kit (Promega Medicine, WI) according to the manufacturer's protocol. 5,000 cells were seeded in 96-well plates and incubated at 37°C for 24-72 hours. Eight wells were used for each clone in each assay. At each time point, 15 µl of dye solution was added to each well and the cells were incubated at 37°C for 4 hours. After incubation, 100 µl solubilization/stop solution were added to each well, and the plates incubated overnight at room temperature. The absorbance was determined at 595 nm using an ELISA plate reader.

Control and hPTTG-overexpressing NIH 3T3 cells were used to perform this assay. The results indicated that the growth of cells transfected with the PTTG-expressing vector was suppressed by 30-45% as compared with cells transfected with blank vector. These results clearly show that the

PTTG protein inhibits cell proliferation.

Example 15: In Vitro and In Vivo Transformation Assay

(a) In vitro transformation assay

5 Control and hPTTG-transfected cells were tested for anchorage-independent growth in soft agar. 3 ml of soft agar (20% of 2X DMEM, 50% DMEM, 10% fetal bovine serum, and 20% of 2.5% agar, melted and mixed at 45°C) were added to 35mm tissue dishes. 10,000 cells were mixed with 1 ml soft agar and added to each dish, and incubated for 2 weeks until colonies could be counted and photographed.

(b) In vivo Transformation Assay

10 5×10^5 cells containing either a blank vector or hPTTG-expressing cells were injected into nude mice. The mice were sacrificed two weeks after injection, and the tumors formed near the injection sites examined.

When the NIH 3T3 cells stably transfected with the PTTG-expressing vector were tested in an anchorage-independent growth assay, these cells caused large colony formation on soft agar, 15 suggesting the transforming ability of PTTG protein.

When the NIH 3T3 cells were injected into nude mice, they caused in vivo tumor formation within 2 weeks after injection. These data indicate that human PTTG, as its rat homologue, is a potent transforming gene.

SUMMARY OF SEQUENCES

20 SEQ ID NO:1 is the nucleic acid sequence (and the deduced amino acid sequence) of cDNA encoding the rat PTTG protein of the present invention.

SEQ ID NO:2 is the deduced amino acid sequence of the rat PTTG protein of the present invention.

25 SEQ. ID No:3 is the nucleic acid sequence of cDNA encoding a human PTTG protein of this invention.

SEQ. ID No:4 is the deduced amino acid sequence of a human PTTG protein of this invention.

30 While the invention has been described in detail with reference to certain preferred embodiments thereof, it should be understood that modifications and variations to the embodiments and exemplary disclosure provided, are within the spirit and scope of the invention as described and claimed in this patent.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Shlomo Melmed and Lin Pei
- (ii) TITLE OF INVENTION: PITUITARY-TUMOR-TRANSFORMING-GENES, AND RELATED PRODUCTS
- (iii) NUMBER OF SEQUENCES: 4
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 - (B) COMPUTER: IBM-PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
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 - (C) REFERENCE/DOCKET NUMBER: FP07 39556
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 213-622-7700
 - (B) TELEFAX: 213-489-4210

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 974 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: both
 - (D) TOPOLOGY: both
- (ii) MOLECULE TYPE: cDNA
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 293..892

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

AATTTCGGCAC GAGCCAACCT TGAGCATCTG ATCCTCTTGG CTTCTCCTTC CTATCGCTGA

GCTGGTAGGC	TGGAGACAGT	TGTTTGGGTG	CCAACATCAA	CAAACGATTT	CTGTAGTTTA	120
GCGTTTATGA	CCCTGGCGTG	AAGATTTAAG	GTCTGGATTA	AGCCTGTTGA	CTTCTCCAGC	180
TACTTCTAAA	TTTTTGTGCA	TAGGTGCTCT	GGTCTCTGTT	GCTGCTTAGT	TCTTCCAGCC	240
TTCCTCAATG	CCAGTTTTAT	AATATGCAGG	TCTCTCCCCT	CAGTAATCCA	GG ATG	295
					Met	
					1	
GCT ACT CTG ATC TTT GTT GAT AAG GAT AAC GAA GAG CCA GGC AGC CGT						343
Ala Thr Leu Ile Phe Val Asp Lys Asp Asn Glu Glu Pro Gly Ser Arg	5	10		15		
TTG GCA TCT AAG GAT GGA TTG AAG CTG GGC TCT GGT GTC AAA GCC TTA						391
Leu Ala Ser Lys Asp Gly Leu Lys Leu Gly Ser Gly Val Lys Ala Leu	20	25		30		
GAT GGG AAA TTG CAG GTT TCA ACG CCA CGA GTC GGC AAA GTG TTC GGT						439
Asp Gly Lys Leu Gln Val Ser Thr Pro Arg Val Gly Lys Val Phe Gly	35	40		45		
GCC CCA GGC TTG CCT AAA GCC AGC AGG AAG GCT CTG GGA ACT GTC AAC						487
Ala Pro Gly Leu Pro Lys Ala Ser Arg Lys Ala Leu Gly Thr Val Asn	50	55		60	65	
AGA GTT ACT GAA AAG CCA GTG AAG AGT AGT AAA CCC CTG CAA TCG AAA						535
Arg Val Thr Glu Lys Pro Val Lys Ser Ser Lys Pro Leu Gln Ser Lys	70	75		80		
CAG CCG ACT CTG AGT GTG AAA AAG ATC ACC GAG AAG TCT ACT AAG ACA						583
Gln Pro Thr Leu Ser Val Lys Lys Ile Thr Glu Lys Ser Thr Lys Thr	85	90		95		
CAA GGC TCT GCT CCT GCT CCT GAT GAT GCC TAC CCA GAA ATA GAA AAG						631
Gln Gly Ser Ala Pro Ala Pro Asp Asp Ala Tyr Pro Glu Ile Glu Lys	100	105		110		
TTC TTC CCC TTC GAT CCT CTA GAT TTT GAG AGT TTT GAC CTG CCT GAA						679
Phe Phe Pro Phe Asp Pro Leu Asp Phe Glu Ser Phe Asp Leu Pro Glu	115	120		125		
GAG CAC CAG ATC TCA CTT CTC CCC TTG AAT GGA GTG CCT CTC ATG ATC						727
Glu His Gln Ile Ser Leu Leu Pro Leu Asn Gly Val Pro Leu Met Ile	130	135		140	145	
CTG AAT GAA GAG AGG GGG CTT GAG AAG CTG CTG CAC CTG GAC CCC CCT						775
Leu Asn Glu Glu Arg Gly Leu Glu Lys Leu Leu His Leu Asp Pro Pro	150	155		160		
TCC CCT CTG CAG AAG CCC TTC CTA CCG TGG GAA TCT GAT CCG TTG CCG						823
Ser Pro Leu Gln Lys Pro Phe Leu Pro Trp Glu Ser Asp Pro Leu Pro	165	170		175		
TCT CCT CCC AGC GCC CTC TCC GCT CTG GAT GTT GAA TTG CCG CCT GTT						871
Ser Pro Pro Ser Ala Leu Ser Ala Leu Asp Val Glu Leu Pro Pro Val	180	185		190		
TGT TAC GAT GCA GAT ATT TAAACGTCTT ACTCCTTTAT AGTTTATGTA						919
Cys Tyr Asp Ala Asp Ile	195	200				
AGTTGTATTA ATAAAGCATT TGTGTGTAAA AAAAAAAAAA AAAACTCGAG AGTAC						974

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 199 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

Met Ala Thr Leu Ile Phe Val Asp Lys Asp Asn Glu Glu Pro Gly Ser
 1           5           10           15
Arg Leu Ala Ser Lys Asp Gly Leu Lys Leu Gly Ser Gly Val Lys Ala
      20           25           30
Leu Asp Gly Lys Leu Gln Val Ser Thr Pro Arg Val Gly Lys Val Phe
      35           40           45
Gly Ala Pro Gly Leu Pro Lys Ala Ser Arg Lys Ala Leu Gly Thr Val
      50           55           60
Asn Arg Val Thr Glu Lys Pro Val Lys Ser Ser Lys Pro Leu Gln Ser
      65           70           75           80
Lys Gln Pro Thr Leu Ser Val Lys Lys Ile Thr Glu Lys Ser Thr Lys
      85           90           95
Thr Gln Gly Ser Ala Pro Ala Pro Asp Asp Ala Tyr Pro Glu Ile Glu
      100           105           110
Lys Phe Phe Pro Phe Asp Pro Leu Asp Phe Glu Ser Phe Asp Leu Pro
      115           120           125
Glu Glu His Gln Ile Ser Leu Leu Pro Leu Asn Gly Val Pro Leu Met
      130           135           140
Ile Leu Asn Glu Glu Arg Gly Leu Glu Lys Leu Leu His Leu Asp Pro
      145           150           155           160
Pro Ser Pro Leu Gln Lys Pro Phe Leu Pro Trp Glu Ser Asp Pro Leu
      165           170           175
Pro Ser Pro Pro Ser Ala Leu Ser Ala Leu Asp Val Glu Leu Pro Pro
      180           185           190
Val Cys Tyr Asp Ala Asp Ile
      195

```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 779 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: both

(D) TOPOLOGY: both

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 293..892

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```

1  ATGGCCGCGA GTTGTGGTTT AAACCAGGAG TGCCGCGCGT CCGTTCACCG
51  CGGCCTCAGA TGAATGCGGC TGTTAAGACC TGCAATAATC CAGAATGGCT
101 ACTCTGATCT ATGTTGATAA GGAAAATGGA GAACCAGGCA CCCGTGTGGT
151 TGCTAAGGAT GGGCTGAAGC TGGGGTCTGG ACCTTCAATC AAAGCCTTAG
201 ATGGGAGATC TCAAGTTTCA ACACCACGTT TTGGCAAAAC GTTCGATGCC
251 CCACCAGCCT TACCTAAAGC TACTAGAAAG GCTTTGGGAA CTGTCAACAG
301 AGCTACAGAA AAGTCTGTAA AGACCAAGGG ACCCCTCAAA CAAAAACAGC
351 CAAGCTTTTC TGCCAAAAAG ATGACTGAGA AGACTGTTAA AGCAAAAAGC
401 TCTGTTCTTG CCTCAGATGA TGCCTATCCA GAAATAGAAA AATTCTTTCC
451 CTTCAATCCT CTAGACTTTG AGAGTTTGA CCTGCCTGAA GAGCACCAGA
501 TTGCGCACCT CCCCTTGAGT GGAGTGCCTC TCATGATCCT TGACGAGGAG
551 AGAGAGCTTG AAAAGCTGTT TCAGCTGGGC CCCCCTTCAC CTGTGAAGAT
601 GCCCTCTCCA CCATGGGAAT CCAATCTGTT GCAGTCTCCT TCAAGCATTC
651 TGTCGACCCT GGATGTTGAA TTGCCACCTG TTTGCTGTGA CATAGATATT
701 TAAATTTCTT AGTGCTTCAG AGTTTGTGTG TATTTGTATT AATAAAGCAT
751 TCTTTAACAG ATAAAAA AAAA

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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 202 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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1  MATLIYVDKE NGEPGTRVVA KDGLKLGSGP SIKALDGRSQ VSTPRFGKTF
51  DAPPALPKAT RKALGTVNRA TEKSVKTKGP LKQKQPSFSA KKMTEKTVKA
101 KSSVPASDDA YPEIEKFFPF NPLDFESFDL PEEHQIAHLP LSGVPLMILD
151 EERELEKLFQ LGPPSPVKMP SPPWESNLLQ SPSSILSTLD VELPPVCCDI
201 DI*

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CLAIMS

1. A purified, isolated pituitary-tumor-transforming-gene (PTTG) polypeptide expressed by pituitary tumor cells, which binds anti-PTTG antibody.
2. The polypeptide of claim 1, comprising the amino acid sequence encoded by the coding regions of SEQ. ID No: 1, of SEQ. ID No: 3, and of fragments thereof 5 to 50 amino acids long which bind anti-PTTG antibody.
3. The polypeptide of claim 2, comprising the amino acid sequence encoded by the coding region of SEQ. ID No: 1.
4. The polypeptide of claim 3, comprising nucleotides 292 to 899 of SEQ. ID No: 1.
5. The polypeptide of claim 2, comprising fragments 5 to 50 amino acids long of the amino acid sequence encoded by the coding region of SEQ. ID No: 1.
6. The polypeptide of claim 2, comprising the amino acid sequence encoded by the coding sequence of SEQ ID No: 3.
7. The polypeptide of claim 6, comprising the amino acid sequence encoded by nucleotides 292 to 899 of SEQ. ID No: 3.
8. The polypeptide of claim 6, comprising fragments 5 to 50 amino acids long of the amino acid sequence encoded by the coding region of SEQ. ID No: 3.
9. The polypeptide of claim 2, comprising an amino acid sequence selected from the group consisting of SEQ. ID No: 2, SEQ. ID No: 4 and fragments thereof 5 to 50 amino acids long which bind anti-PTTG antibody.
10. The polypeptide of claim 9, comprising SEQ. ID No: 2.
11. The polypeptide of claim 9, comprising SEQ ID NO: 4.
12. The polypeptide of claim 9, comprising fragments of SEQ. ID No: 2 5 to 50 amino acids long which bind anti-PTTG antibody.
13. The polypeptide of claim 9, comprising fragments of SEQ. ID No: 4 5 to 50 amino acids long which bind anti-PTTG antibody.
14. The polypeptide of claim 1, which is of mammalian origin.
15. The polypeptide of claim 14, wherein its origin is selected from the group consisting of human, rat, mouse, porcine, ovine, canine, human and bovine origins.
16. A composition, comprising the polypeptide of claim 1, and a carrier.
17. An isolated, purified polynucleotide, comprising a nucleic acid encoding the polypeptide of claim 1.
18. The polynucleotide of claim 17, comprising a nucleic acid selected from the group consisting of SEQ. ID No: 1, SEQ. ID No: 3, and fragments thereof 10 to 150 nucleotide long.
19. The polynucleotide of claim 17, comprising a nucleic acid selected from the group consisting of (a) a nucleic acid selected from the group consisting of those encoding SEQ ID

NO:2, SEQ. ID No: 4, and fragments thereof 5 to 50 amino acids long binding anti-PTTG antibody;

(b) splice variant sequences thereof;

(c) probe/primer fragments thereof; and

(c) nucleic acid that hybridizes to the nucleic acid of (a) under moderately stringent conditions.

20. The polynucleotide of claim 17, comprising a nucleic acid selected from the group consisting of those that hybridize under high stringency conditions to the PTTG coding region of SEQ. ID No:1.

21. The polynucleotide of claim 20, comprising a fragment 10 to 150 nucleotide long that hybridizes to a portion of the PTTG coding region of SEQ. ID No:1.

22. The polynucleotide of claim 19, comprising the PTTG coding region of SEQ ID NO:1.

23. The polynucleotide of claim 19, comprising the PTTG coding region of SEQ. ID No: 3.

24. The polynucleotide of claim 23, comprising fragments 10 to 150 nucleotide long of SEQ. ID No: 3.

25. The polynucleotide of claim 17, which is an RNA.

26. The polynucleotide of claim 17, which is a DNA.

27. A vector comprising the polynucleotide of claim 17.

28. A composition, comprising the vector of claim 27, and a carrier.

29. A host cell carrying the polynucleotide of claim 17.

30. A host cell carrying the vector of claim 25.

31. A composition, comprising the host cell of claim 29, and a carrier.

32. An oligonucleotide comprising at least 15 nucleotides which hybridize under stringent conditions to the nucleic acid comprised in claim 17.

33. The oligonucleotide of claim 32, hybridizing under stringent conditions to SEQ ID No: 1.

34. The oligonucleotide of claim 32, hybridizing under stringent conditions to SEQ ID No: 3.

35. The oligonucleotide of claim 32, further comprising a detectable label or marker.

36. The oligonucleotide of claim 32, which is an RNA.

37. The oligonucleotide of claim 32, which is a DNA.

38. An antisense oligonucleotide capable of hybridizing under stringent conditions to mRNA corresponding to the nucleic acid comprised in claim 17.

39. A composition, comprising the antisense oligonucleotide of claim 38, and a carrier.

40. A PTTG diagnostic kit, comprising at least one oligonucleotide of claim 32, and

instructions for its use in the detection of PTTG encoding DNA.

41. A method of producing a PTTG polypeptide, comprising culturing the host cells of claim 30, in an expression medium and under effective expression conditions;

allowing the PTTG protein to be expressed and accumulate in the medium; and separating the medium comprising the PTTG protein from the cells.

42. The method of claim 41, further comprising separating the PTTG protein from the medium and any remaining components.

43. An isolated anti-PTTG antibody, selectively binding the polypeptide of claim 1.

44. The antibody of claim 43, which is a monoclonal antibody.

45. The antibody of claim 44, which is a polyclonal antibody.

46. A composition, comprising the antibody of claim 43, and a carrier.

47. A composition, comprising an amount of the antisense oligonucleotide according to claim 32, effective to inhibit expression of a human PTTG gene, and a pharmaceutically acceptable hydrophobic carrier which passing through a cell membrane.

48. A transgenic non-human mammal, carrying at least one non-native polynucleotide expressing a PTTG protein.

49. The transgenic non-human mammal of claim 48, carrying the polynucleotide of claim 17.

50. The transgenic non-human mammal of claim 48, carrying a mutated PTTG encoding nucleic acid, which expresses a non-native PTTG protein.

51. The transgenic non-human mammal of claim 48, which is a mouse.

52. A method of identifying mammalian PTTG nucleic acid, comprising contacting the nucleic acid of a mammalian sample with the oligonucleotide of claim 32 in labeled form, under stringent hybridization conditions;

detecting the presence of a label in the hybridized nucleic acid of the sample; and

identifying the presence of a mammalian nucleic acid in the presence of label in the nucleic acid of the mammalian sample.

53. A method of isolating mammalian PTTG nucleic acid, comprising the method of claim 52; and

separating the labeled hybridized nucleic acid from unlabeled nucleic acid from the mammalian sample.

54. A method of determining the presence of human PTTG peptide in a sample, comprising

contacting a human sample with the antibody of claim 43 and allowing the formation of a complex between the antibody and any PTTG peptide present in the sample;

detecting the presence of any antibody-PTTG complex; and
determining that a human PTTG peptide if any PTTG peptide-antibody complex is present in the sample.

55. The ~~method~~ of claim 54, wherein the antibody carries a detectable label, and the detecting step is carried out by detecting the antibody label after separation of the labeled complex from unlabeled and labeled antibody.

56. Single strand PTTG amplification primers/probes, comprising a nucleic acid sequence corresponding to a nucleic acid selected from the group consisting of SEQ. ID No: 1, SEQ. ID No: 3, and fragments thereof 10 to 150 nucleotide long.

57. A method for detecting a pathological mass associated with PTTG expression, comprising detecting in a subject's cells a transcribed or mutant nucleic acid of a sequence corresponding to the polynucleotide of claim 17.

58. A PTTG detection kit, comprising the antibody of Claim 43; and instructions for its use.

59. The kit of claim 58, further comprising a Pituitary-Tumor-Transforming-gene polypeptide selectively bound by the antibody.

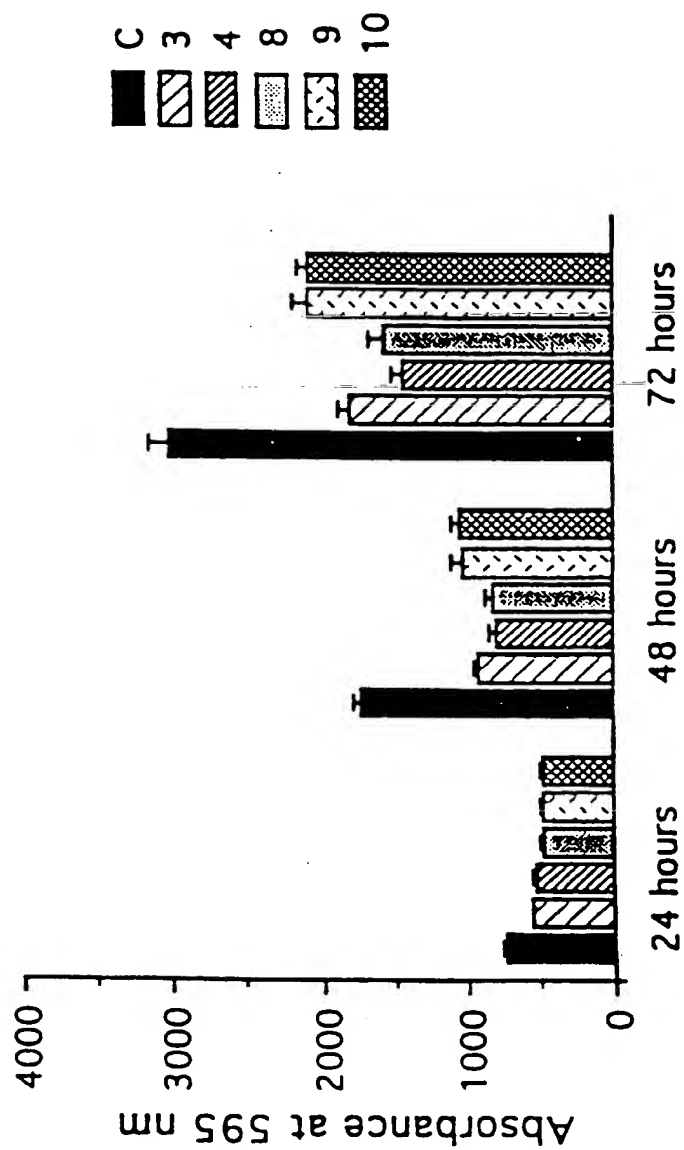


FIGURE 1

